PROCEEDINGS OF FIFTH CONFERENCE ON PROCESSING AS RELATED TO NUTRITIVE VALUE OF COTTONSEED MEAL

JANUARY 19-20, 1959
NEW ORLEANS, LA.

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SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
AND
EDUCATIONAL SERVICE AND RESEARCH COMMITTEE, NATIONAL COTTONSEED PRODUCTS ASSOCIATION

Southern Utilization Research and Development Division
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This report contains the proceedings of the Research Planning Conference on Processing as Related to Nutritive Quality of Cottonseed Meals, sponsored jointly by the Agricultural Research Service's Southern Utilization Research and Development Division and the Educational Service and Research Committee of the National Cottonseed Products Association. The conference was held January 19-20, 1959, at the Southern Regional Research Laboratory, Southern Utilization Research and Development Division.

The purpose of this conference was to develop plans for cooperative feeding studies to determine the nutritive qualities of various cottonseed meals in rations for broilers, laying hens, and swine by cooperating federal, state, and industrial laboratories. The results are necessary to provide information concerning the properties of cottonseed meals which affect nutritive quality and the effects of seed processing conditions on those properties. This information is of utmost importance to the cottonseed crushing industry in its endeavor to upgrade the quality of cottonseed meal for increased utilization in animal rations.

Abstracts of papers presented at the conference, discussions of results among conference participants, and research proposed for cooperative feeding studies are presented. More details may be obtained by writing the Southern Utilization Research and Development Division, P. O. Box 7307, New Orleans 19, Louisiana, or to persons listed on the program.

The use of trade names in this report is for identification and implies no endorsement by the Department of Agriculture of the product over similar products not mentioned.

CONTENTS

General Chairman -- T. H. Hopper

OPENING REMARKS

T. H. Hopper
Southern Utilization Research and Development Division
New Orleans, Louisiana

We welcome you today and hope that the results from this conference will be of benefit to you and the cottonseed industry.

Dr. Fisher has asked us to welcome you and tell you he is sorry that he cannot be present. He departed last week for South America as a member of a research team. Dr. Goheen, Assistant Director of Southern Division, will greet you in Dr. Fisher's absence.

WELCOMING ADDRESS

G. E. Goheen, Assistant Director
Southern Utilization Research and Development Division
New Orleans, Louisiana

It is a pleasure for me to welcome members of this conference for Dr. Fisher and the Southern Division. You should be proud of the progress made in cottonseed meal nutrition knowledge. It has been good. The subject of today's conference is a timely one. We have many problems to tackle at this meeting. There are always complex problems involved in the utilization of farm crops. The subject of cottonseed meal utilization is one in which fullest cooperation is needed. We at this Laboratory are glad to be a member of your cooperating team. Your attendance at this meeting is appreciated.

You are extended an invitation to visit our laboratories and talk with our scientists. Our work is not only concerned with cotton and cottonseed, but also involves research on rice, peanuts, cottonseed oil, naval stores, sugarcane, fruits and vegetables. In the past year we have listed 24 developments, 134 publications, 16 patents, and 25 applications for new patents. We are proud of this progress.

We are glad that you are here with us, and we hope that this meeting will point up both problems and progress in their solution.

RESPONSE

G. A. Harper, Director Educational Service
National Cottonseed Products Association
Dallas, Texas

It is a pleasure to attend this meeting, and I wish to extend the appreciation of the National Cottonseed Products Association for the help given by you in making this conference possible. Our thanks are extended also to the experiment stations, universities, and other groups for their efforts in this undertaking and those of past conferences. The National Cottonseed Products Association is very interested and concerned with today's meeting. We have with us today our President and Directors of our Research Committee and of our Cottonseed Meal Committee. We are happy to be here.

CURRENT COTTONSEED RESEARCH PROGRAM

A. M. Altschul
Southern Utilization Research and Development Division
New Orleans, Louisiana

I think this has been a distinguished series of conferences on increasing the utilization of cottonseed meal and the effects of seed processing conditions on meal nutritive qualities. The summaries of status of investigations which have preceded each conference and the summaries of results of conferences have been very instructive.

The object of this conference is to summarize the status of results as we see them. We must bring together the results of our investigations with cottonseed meals and find out what remains to be done. We no longer need to assume an exploratory program on cottonseed meal nutrition, but can arrive at definite points of contact for future investigations. All problems may not be solved, but we will reach a point in which many will have been solved. For example, if we assume a certain lysine content in cottonseed protein, then the whole program may be planned on that average value of lysine in the seed protein. I am working with seed proteins. My group, the Seed Protein Laboratory, may discover some results that will be beneficial to you. For example, if a protein is found to contain 10-12% arginine, and one fraction of the protein may be found to contain 80% of that amount of total arginine, it will be of great benefit to you to know what fraction contains the most arginine. Thenaa study can be undertaken to determine the effects of seed processing conditions on a particular protein fraction. Investigations of this type may prove most helpful. If, for example, it is found that the carbohydrate exists in one part of the seed and the aminopolysaccharide exists in another, the effects of seed processing conditions to each seed fraction will be different. Our studies with seed proteins show that the seed protein fractions are not of the same

or average composition. Part of the protein may be combined chemically in the seed with lipoid material and part with carbohydrate. We have been investigating the subcellular particles of the peanut, and have been able to perfect the separation of aleurone grains and other particles. This may lead to separation of a carbohydrate-free protein fraction.

You, as researchers in this field, may have to decide on a new approach for solution of the problems concerned with cottonseed meal nutritive quality. In future work on cottonseed, we may talk not only of pigment gland separation but may talk in terms of cottonseed protein fractionation. There is a great need for knowing the cytological arrangement of constituents in the cottonseed and the effects of seed processing conditions on the chemistry of those constituents.

I am glad to see all of you and extend an invitation to visit our Seed Protein Laboratories and talk to our members. I wish you success in this conference.

PURPOSE OF CONFERENCE

T. H. Hopper
Southern Utilization Research and Development Division
New Orleans, Louisiana

It has been two years since our last meeting and changes have been made in the utilization of cottonseed meal for swine and poultry rations. Your purpose here is to review the results presented by participating members and formulate plans for future work including collaborative feeding studies. Three panels have been set up. These are: Panel on cottonseed meal utilization in broiler rations; panel on cottonseed meal in rations for laying hens; and panel on cottonseed meal in swine rations. Each panel has an appointed presiding officer. The presiding member will call on each member of his committee to make a report. We want the committees of this conference to meet separately and review the results presented at the session of his particular panel discussion and make recommendations for future research and for carrying out of the collaborative feeding studies. The studies are to be undertaken as soon as possible to determine the nutritive qualities of various cottonseed meals for swine and poultry. Dr. H. E. Robinson is to preside at the panel on cottonseed meal in broiler rations, and will be the first to be heard by you. This afternoon Dr. H. L. Wilcke will preside at the second panel discussion. Dr. C. M. Lyman will preside at the panel on use of cottonseed meal in swine rations which is scheduled for tomorrow morning's meeting. We will have the first technical report of this conference. It will be presented by Dr. Tillman.

Response of Ruminants to Cottonseed Meals of Known Quality as Per Chick Test

Allen D. Tillman
Oklahoma State University
Stillwater, Oklahoma

Woods and Tillman reported that either low (39.6%) or high (70.1%) nitrogen soluble cottonseed meal supported less gain and required more feed and protein per unit of gain than sesame or a combination of soybean-sesame meals. Further research by these workers indicated that both of the cottonseed meals furnished protein of lower digestibility than that of the other protein supplements.

When the results of the collaborative study of commercially processed meals were published, the writer obtained enough of two meals used in this study for further research on this problem. One meal, cottonseed meal #13, was processed in a high-speed screw-press and was the poorest meal in that study. The other meal, cottonseed meal #45 was a prepress, solvent-extracted product and was one of the better meals. Each cottonseed meal and commercial soybean meal were individually included in semisynthetic and isonitrogenous rations for sheep. The rations contained alpha cellulose, cottonseed hulls, corn oil, corn sugar, corn starch, minerals, Vitamins A and D, and the protein source, thus each protein source supplied over 90 percent of the dietary protein. The results, presented in Table 1, represent the average of 10 animals per treatment. The

Table 1. Percentage Digestibility of Ration Components of Sheep Receiving Cottonseed Meal 13, Cottonseed Meal 45 or Soybean Meal.

Ration	Organic Matter	Crude Protein	Crude Fiber	NFE
CSM 13	72.0	41.8	67.7	78.7
CSM 45	73.5	47.2^{t}	69.6	79.3
Soybean Meal	76.2^{t}	53.7**	73.8*	81.0

t signifies $P = 0.07$ in both cases
* signifies $P \leq 0.05$
** signifies $P \leq 0.01$

protein of soybean meal was better digested than that of either cottonseed meal; the differences between soybean meal and CSM 13 and CSM 45 being significant statistically ($P \leq 0.01$ and $P \leq 0.05$, respectively). The digestion coefficients of the protein supplied by CSM 13 and 45 were 41.8 and 47.2, respectively ($P = 0.07$). In growth trials involving ten

individually-fed animals per group, two cottonseed meals and soybean meal were separately included in a ration composed of cottonseed hulls, cane molasses, corn sugar, corn starch, corn oil, salt, limestone, dicalcium phosphate, Vitamins A and D. All rations were isonitrogenous and were fed to sheep for 56 days. The cottonseed meals used in ration 1 (SCO) were obtained from the Southern Cotton Oil Company, Memphis, Tennessee, and essentially was cottonseed meal 13, while the other cottonseed meal was obtained from the Ranchers Cotton Oil Company, Fresno, California. Soybean meal was obtained commercially. Results of this trial are presented in Table 2. Soybean meal apparently pro-

Table 2. Feeding Trial Results Obtained with Lambs Receiving Two Different Cottonseed Meals or Soybean Meal. (Sufficient Energy)

Treatment	Daily Gains Lb.	Feed Efficiency Lb.	Protein Efficiency Lb.[a]
CSM (SCO)	0.39	9.8	0.78
CSM (RCO)	0.41	9.5	0.75
SBM (Staley)	0.43[t]	8.6*	0.69*

a Units of protein to produce a unit of gain
* Different from ration 1 ($P \leq 0.05$)

moted slightly faster gains than either of the meals and greater efficiency of feed and protein utilization. Differences between cottonseed meals were small and statistically insignificant. It is noted, however, that the trend in all growth trials conducted in this laboratory have been in the same direction; the cottonseed meals of higher nitrogen solubility and ones showing best results in chick growth tests have given slightly better results in tests with sheep. A second growth trial with the same protein meals was conducted. Each meal furnished all of the supplemental protein in a wintering type ration for sheep, the roughage being cottonseed hulls. The design and results of this trial are shown in Table 3. Again, the trends noted in previous trials are found in the present experiment. It should be pointed out that the ration used in the present trial supplied a liberal quantity of protein while in all previous growth trials conducted in this laboratory, protein was the limiting nutrient. In a similar design English workers showed that casein was a poor protein supplement for ruminants. Further results by that group indicated that the protein of casein was easily degraded by rumen microflora resulting in higher ruminal ammonia content as well as higher plasma nonprotein nitrogen values and subsequent higher urinary loss. In feeding trials involving casein plus a low-quality hay, casein was ineffective in maintaining body weight. Various heat treatments which made the protein of casein less soluble resulted in lowered

Table 3. Feeding Trial Results Obtained with Lambs Receiving Two Different Cottonseed Meals or Soybean Meal. (Insufficient Energy)

Item	CSM (SCO)	CSM (RCO)	SBM Staley
Daily protein supplement, lb.	0.58	0.50	0.50
Daily CSH intake, lb.	2.48	2.51	2.45
Daily Gains, lb.	0.14	0.14	0.18*
Feed Efficiency, lb.	19.10	18,80	14.50*
Protein Efficiency, lb.	1.00	0.99	0.76*

* $P \leq 0.05$

ruminal ammonia values, lowered plasma nonprotein nitrogen values, lowered urinary nitrogen loss, and gain in body weight. They suggested that for ruminants, treatments such as heat might make a protein more desirable presumably because it would slow down proteolysis by microbial enzymes of the rumen. The results of the present experiment indicate that the heat treatment used in the preparation of this particular soybean meal might have been high enough to attain this property; however, that point has not been studied. In looking over some of the earlier nitrogen balance results obtained in this laboratory, the writer found results which would indicate that some soybean meals contain protein that is too soluble for efficient utilization by this animal. In several experiments the protein of soybean meal was much better digested than that of cottonseed meal but subsequent urinary loss was much greater, thus the cottonseed protein supported greater nitrogen retention. This field needs further and more critical study.

Artificial Rumen Studies

The artificial rumen studies were designed to compare protein sources and to study the effect of various heat treatments on these proteins. Digestibility by microflora of added cellulose was the criteria. The protein source under test supplied less nitrogen than needed for optimum digestibility of cellulose and the protein sources supplied the same level of nitrogen. The results of the first trial are shown in Table 4. In this trial the protein of CSM #45 promoted greater cellulose digestibility than CSM #13. The results of the second trial are shown in Table 5. The solvent-extracted meals promoted greater cellulose digestibility than those meals which were produced by screw-presses. It is also apparent that the high-speed, screw-pressed meal supported less cellulose digestibility than the meals produced by screw-press run at

Table 4. Effect of Protein Source upon Cellulose Digestibility by Rumen Microflora in vitro.

Protein Source	Cellulose Digestibility
CSM 13	35
CSM 45	54**
CSM (Fiberless)	48

** $P \leq 0.01$

Table 5. Effect of Protein Source Upon Cellulose Digestibility by Rumen Microflora in vitro (Second Trial).*

Protein Source	Nitrogen Sol. %	Cellulose Digestibility %
CSM 6 - Prepress solvent	68	54
CSM 49 - Solvent - degossypolized	84	55
CSM 13 - High speed screw-press	30	16
CSM 21 - Low speed screw-press	47	40
CSM 36 - Low speed screw-press	39	27

* Note: At the request of the Southern Utilization Research and Development Division, The Southern Cotton Oil Company made a deliberate effort to produce a few tons of cottonseed meal that would have the properties of Cottonseed Meal CM-13. This heat damaged meal was used in the experiments reported by Dr. Tillman, and identified by him as SCO meal.

a lower speed. Table 6 exhibits the results obtained when the various protein sources were heated. Autoclaving for 45 minutes and application of dry heat for 1-1/2 hours at 100° C. reduced the ability of soybean meal to support cellulose digestion by rumen microflora; this being also true of CSM-49 and the CSM obtained from Ranchers Cotton Oil Company. Autoclaving for 15 minutes and application of dry heat for 1-1/2 hours at 80° C. also similarly effective with CSM-49 and the RCO product; however, none of the heat treatments had any effect upon CSM-13.

Table 6. The Effect of Heating of Various Protein Supplements upon the Digestibility of Cellulose by Rumen Microflora (Third Trial).

Treatments	Cellulose Digestibility			
	SBM	CSM-49	CSM-13	CSM-RCO
Untreated	50	34	24	33
Autoclaved				
5 min.	--	27	24	25
15 min.	--	24	23	24
45 min.	36	23	24	22
Dry Heat - 1-1/2 hrs.				
60° C.	--	35	23	34
80° C.	--	28	21	25
100° C.	33	21	23	18

Discussion:

Q. What was the method used for determination of cellulose digestibility.

A. It was an in vitro method. The isonitrogenous source (meal) was put in fermentation flask. Cellulose was added and the solubility of cellulose was then determined. If the protein was not available, the organisms could not grow.

Q. Was the effect of free fatty acid content of meal on cellulose digestibility considered.

A. No. We used cottonseed meals as received and did not analyze them for free fatty acid. Free fatty acid may have been a factor, but it was not studied.

Q. Was the Ranchers Cotton Oil Company meal especially prepared for this nutrition study, and therefore, superior in nutritive quality to the meals usually produced at this mill.

A. The meal was a production meal and is typical of their commercially prepared meals.

RESEARCH ON THE CHEMISTRY OF GOSSYPOL[1/]

D. A. Shirley
University of Tennessee
Knoxville, Tennessee

SUMMARY

Gossypol, the principal cottonseed pigment, must be chemically altered or removed during the processing of cottonseed for most uses. The large amount of gossypol potentially available, the reactive nature of the molecule, and its deleterious effects in cottonseed processing are the principal reasons for the importance of the study of the chemical reactions and reaction products of gossypol. Major attention to gossypol chemistry by most workers has thus far been directed toward elucidation of the structure of the molecule, and many years of work on this problem recently culminated in the total synthesis of gossypol by J. D. Edwards.

Continuing work at the University of Tennessee involves a study of miscellaneous new chemical reactions of gossypol and the properties of the products of these reactions. Past and current work has resulted in the accomplishments cited below.

(1) Reduction of gossypol with lithium aluminum hydride yields two new products, methylapogossypol hexaacetate and desoxygossypol tetraacetate. The former compound was converted to methylapogossypolone tetraacetate and methylapohydrogossypolone octaacetate.

(2) Aliphatic amines were found to form stable anils with gossypol. About twenty-five new anil derivatives were prepared, most of them from aliphatic amines. The wide variety of amines used was selected on the basis of (1) amines containing other reactive functional groups (2) amines which might impart physiological activity to the resulting gossypol anils and (3) azo dyes containing primary amino groups.

(3) Desapogossypol hexamethyl ether was demethylated to the previously unknown desapogossypol and this converted to desapogossypol hexaacetate, desapogossypolone tetraacetate and desapohydrogossypolone octaacetate.

(4) A Diels-Alder type adduct between gossypol and cyclopentadiene has been obtained.

(5) Study of a variety of substitution reactions (metalation, Friedel-Crafts, nitration, bromination, etc.) of apogossypol hexamethyl ether indicated nonselective introduction of substituents into various ring and side chain positions.

1/ A report of work done under contract with the U. S. Department of Agriculture, under the supervision of the Southern Utilization Research and Development Division, Agricultural Research Service.

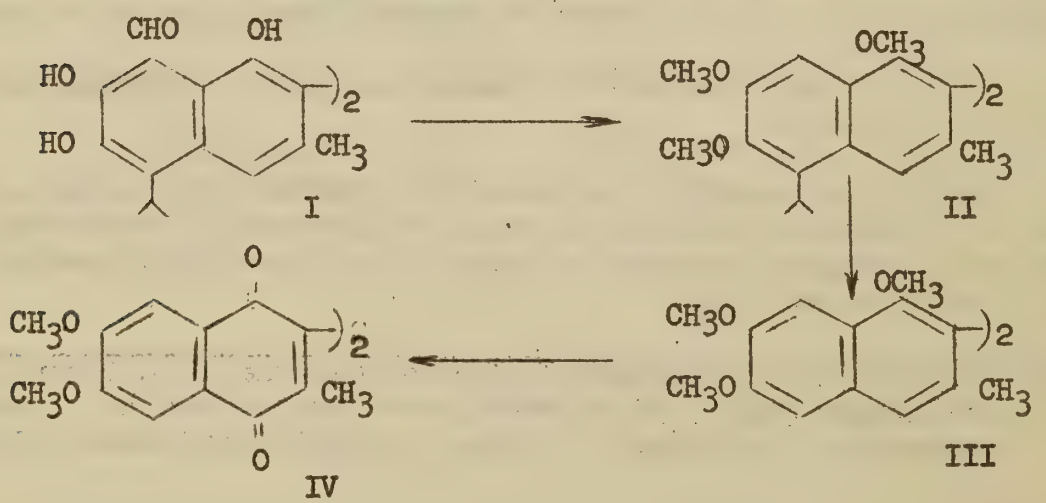
(6) Current study of the oxidation of gossypol with alkaline hydrogen peroxide is designed to provide information on the pathway of oxidative decomposition of gossypol and the products of the decomposition. Two crystalline oxidation products have been isolated and attempts are being made to determine their structures.

The research program for the immediate future will center around continued study of the oxidation of gossypol.

Introduction

The broad objective of this research has been to provide new information on the properties and reactions of gossypol and closely related derivatives of gossypol. Gossypol, the principal pigment of cottonseed, is of economic importance to the cottonseed industry because the pigment must be removed or chemically altered during processing, for most uses, of cottonseed into oil and meal. The amount of gossypol potentially available from the annual United States cottonseed production is about 40,000 tons; consequently, new chemical information about gossypol has potential value in the discovery of new uses for this reactive substance.

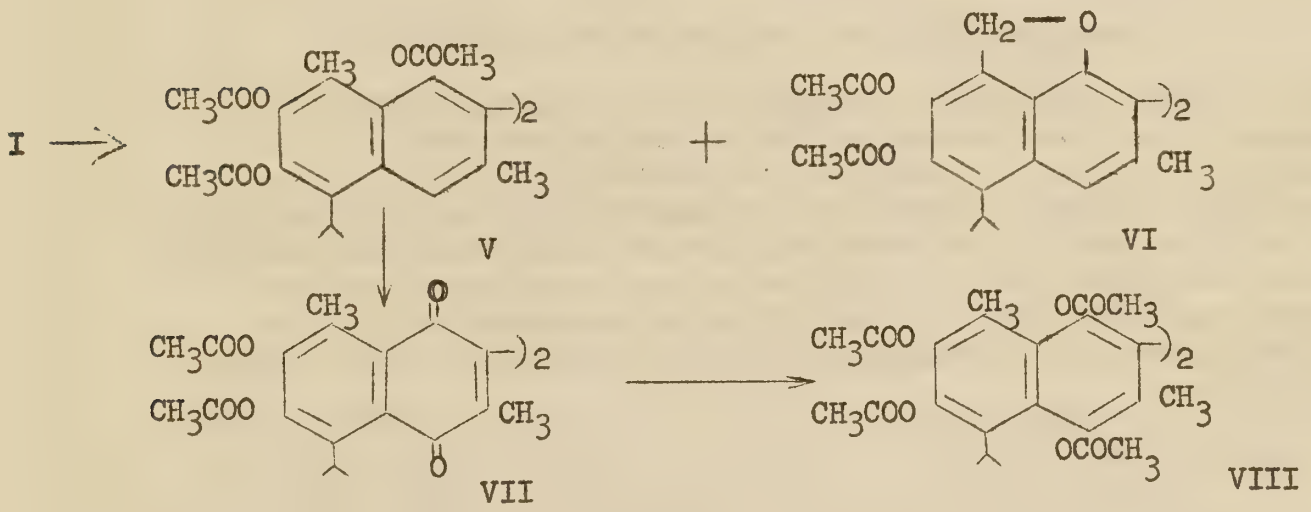
The majority of pure chemical work with gossypol (I) has in the past centered on elucidation of the structure of gossypol. Early work by Carruth and E. P. Clark was followed in the late 1930's by the detailed studies of Roger Adams and his students. This latter work resulted in the postulation of a highly reasonable structure for gossypol (1) and the synthesis of desapogossypolone tetramethyl ether (IV) (2) a degradation product of gossypol. More recently other degradation products have been synthesized which are nearer to the structure of gossypol. These are desapogossypol hexamethyl ether (III) synthesized in our laboratory and apogossypol hexamethyl ether (II) synthesized by Edwards (4). Structural work was culminated in 1958 by the excellent work of Edwards (5) resulting in the formal total synthesis of gossypol and its confirmation of the Adams structure.



While studies of the chemical reactions of gossypol were made as a part of structural investigations, it is apparent from a review of the literature, that there is much room for additional examination of basic chemical reactions of gossypol and closely related gossypol derivatives such as the ones mentioned above (II, III, and IV). Some effort along this line was a part of the work under an earlier (two year) contract between the University of Tennessee and the U. S. Department of Agriculture (1953-1955) and is the principal work of a current three-year contract (1957-1960). It is the purpose of this report to summarize past and present studies on new reactions and reaction products of gossypol and closely related derivatives.

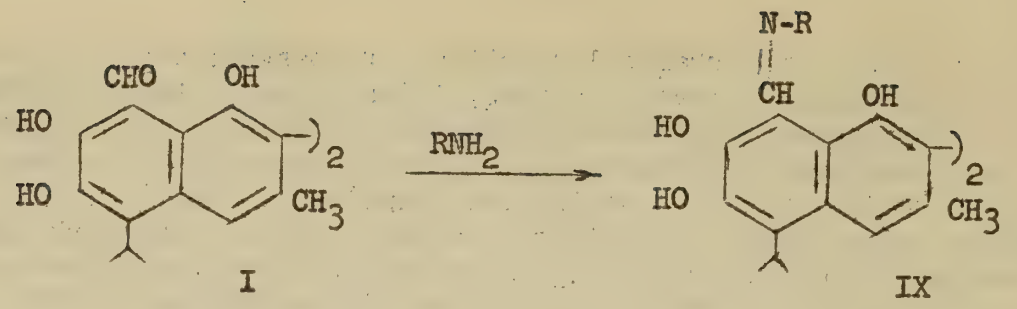
Reduction of Gossypol (6)

The action of lithium aluminum hydride on gossypol (I) followed by acetylation of the unstable product gave two new gossypol derivatives methylapogossypol hexaacetate (V) and desoxygossypol tetraacetate (VI). Reduction of aldehyde to methyl groups observed in the formation of V is an infrequent but not unknown type of action of lithium aluminum hydride. The methylapogossypol hexaacetate (V) was oxidized to methylapogossypolone tetraacetate (VII) which in turn was reduced with zinc in the presence of acetic anhydride to methylapohydrogossypolone octaacetate (VIII).



New Anil Derivatives of Gossypol

During our first contract work period we found that, contrary to some earlier observations, gossypol formed stable anils with aliphatic amines analogous to the long known aromatic anil derivatives (dianilinogossypol, etc.). Initially a series of seven aliphatic anil derivatives were prepared (7); and this has been followed in the present contract work by the synthesis of over fifteen new types, the majority of which are aliphatic and arylaliphatic types (IX). It has been our objective not simply to make new gossypol anils with various amines but to select a

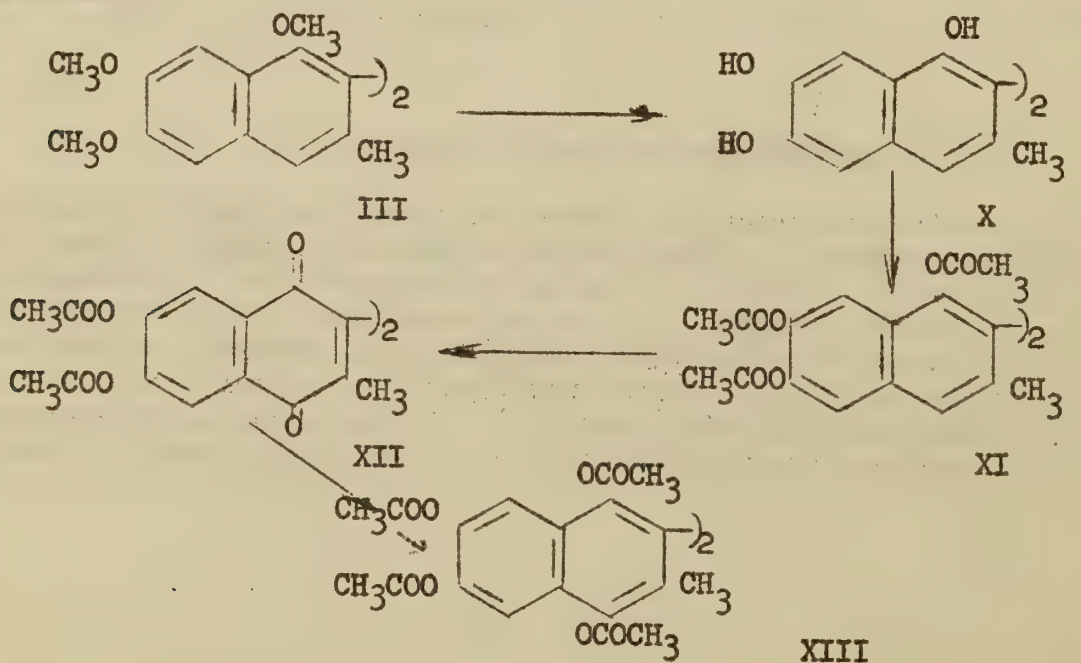


I IX

wide variety of different types of primary amines and to study the scope of the reaction and its applicability to this wide variety of amines. We were particularly interested in (1) amines containing other reactive functional groups which would provide new points of reaction in the resulting anils (2) amines which might impart phyiological activity to the resulting anils and (3) dyes containing primary amino groups which could yield gossypol anils with special dye properties. Some of the amines used are listed under the three preceding categories: (1) allylamine, diethylenetriamine, n-octadecylamine, aminoacetal, p-aminoacetophenone, p-nitrobenzylamine, and p-bromobenzylamine; (2) 3-dimethylaminpropylamine, n-butyl p-aminobenzoate, p-aminobenzenesulfonamide, and β-phenylethylamine; (3) o-tolylazo-o-toluidine and p-aminoazobenzene. In general all the amines used form anils with gossypol in good yield. All products were characterized by elemental analyses.

Desapogossypol and New Derivatives Therefrom

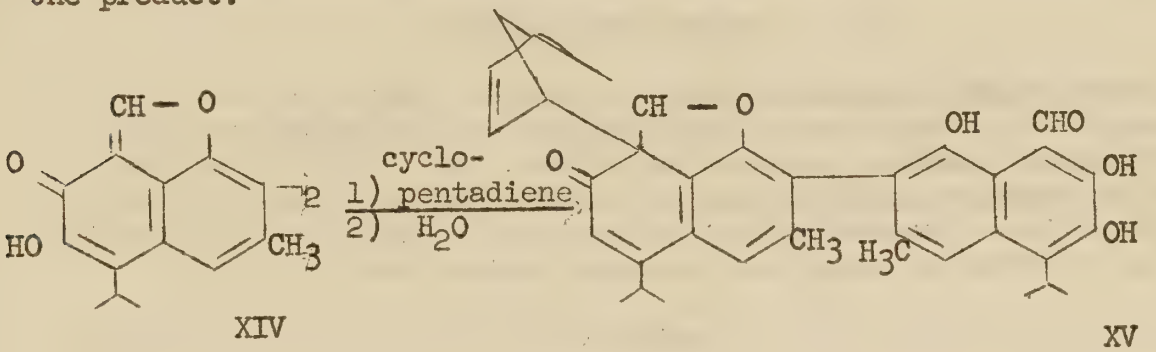
In spite of much attention to desapogossypol hexamethyl ether (III) in connection with the structural investigations referred to in the Introduction, no one previously has prepared the parent type desapogossypol (X). We have recently demethylated desapogossypol hexamethyl ether with pyridine hydrochloride and have obtained desapogossypol (X). This has been converted to the following new derivatives: desapogossypol hexaacetate (XI) desapogossypolone tetraacetate (XII) and desapohydrogossypolone octaacetate (XIII) as indicated in the structures shown.



III X XII XI XIII

Reaction of Gossypol with Conjugated Dienes

The work of Adams (8) first revealed that gossypol and anhydrogossypol (XIV) would form identical adducts with conjugated dienes and 1,3-butadiene and 2,3-dimethyl-1,,3-butadiene were used. We attempted to extend this reaction to other dienes but only with cyclopentadiene could a crystalline adduct be obtained with anhydrogossypol. This adduct showed by analysis to have only one cyclopentadiene molecule per anhydrogossypol molecule and we propose the structure (XV) for the product.



XIV XV

Substitution Reactions of Apogossypol Hexamethyl Ether

Considerable effort has been expended in attempts to form pure products in a variety of common substitution reactions of apogossypol hexamethyl ether. Reactions studied included (1) metalation with alkyllithium and alkylsodium compounds (2) nitration (3) Friedel-Crafts acylation, and (4) bromination. While in all cases good evidence was obtained that substitutuion occurred, in no case could pure products be obtained. The basic difficulty seemed to be that several nuclear and side chain positions are available for substitution and the reactions studies were not or could not be operated so as to substitute certain positions selectively.

The Oxidation of Gossypol

Fundamental to most practical problems associated with the presence of gossypol in cottonseed is the question of the fate of the gossypol under oxidizing conditions. These conditions are present to some degree under many situations in the processing and storing of cottonseed products. There is virtually no prior work on the problem. Oxidative degradation studies used in gossypol structure determination work were carried out on derivatives of gossypol such as gossypol hexamethyl ether and desapogossypol hexamethyl ether rather than the parent molecule.

Some years ago W. H. King of the Southern Utilization Laboratory obtained a colorless product from alkaline peroxide oxidation of gossypol. This material was impure and little work was conducted toward structural characterization. We have recently undertaken a study of this reaction and the reaction product(s) in the hope of discovering the course of the oxidation and the structures of the products.

We have given considerable effort to the problem of purification of the primary oxidation product and optimum conditions for its formation in good yield. The product is acidic and apparently contains both carboxyl and phenolic groups. It has been difficult to purify but recently two crystalline different oxidation products have been obtained as well as an acetate derivative and methyl ester derivative of one of the oxidation products. We are just now getting the complete analytical data, infrared and ultraviolet spectra, etc. which will be used for structure interpretation.

Future Work Program

Our future primary research effort on gossypol chemistry will be the study of the oxidation of gossypol and the oxidation products obtained under various reaction conditions. It is felt that the oxidation reaction deserves careful study because of its practical importance in cottonseed processing problems as well as its potential value as a route to useful new gossypol derivatives.

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Discussion:

Q. Is there any indication that gossypol oxidation products are physiologically inert.

A. Our work has not involved physiological testing. We are negotiating presently with a pharmaceutical company to evaluate the drug action of oxidation products isolated. The samples are tested for activity against micro-organisms such as bacteria, fungi, or viruses.

Q. What are stabilities of compounds you have shown on the blackboard.

A. The anils of gossypol and gossypol hexamethyl ether are stable. Apogossypol is not stable.

Q. Do you have samples of methylated gossypol anils.

A. No. This procedure produces a mixture of products. As yet pure products from a methylated gossypol anil have not been obtained.

Q. Why would you want to methylate gossypol anils.

A. Because of the possible interest in such a compound as a drug or a starting material in preparation of drug.

Q. Has the physiological activity of apogossypol hexamethyl ether been determined.

A. Perhaps the Southern Laboratory has this information from studies of the egg yolk discoloration problem. It is possible that the OH groups of gossypol are needed to produce egg yolk discoloration.

A. When the OH groups of gossypol are tied up, feeding of compound produces no egg yolk discoloration.

HISTOPATHOLOGICAL EFFECT OF GOSSYPOL

T. M. Ferguson and J. R. Couch
Poultry Science Department, Texas Agricultural Experiment Station
College Station, Texas
and R. H. Rigdon, Department of Pathology
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Chronic toxicity symptoms in chickens, as a result of feeding gossypol in the form of pigment glands, include poor growth, emaciation; a reduction in red blood cell count, an enlarged gall bladder, pigmentation of fat and frequently an accumulation of excess fluid in the pericardial and peritoneal cavities. When laying hens are fed pigment glands, containing levels of free gossypol up to 0.4% of the diet, feed intake is reduced and the hens stop laying. Some of the effects of gossypol toxicity may be reduced by feeding higher levels of protein in the ration, as shown by Narain et al. (1957).

It was reported also by our laboratory (Rigdon et al., 1958 a, b) that a hemolytic anemia occurs in young chicks fed pigment glands. It was noted that there was an accumulation of a yellowish-brown pigment in the gossypol-fed chicks. Histochemical tests demonstrated that the pigment was of a ceroid type.

Acute toxicity symptoms in chickens and laying hens were determined by feeding gossypol glands in capsules. Death occurred within 24-65 hours,

feed and water intake was reduced or stopped, and a hemocentration resulted. The free gossypol required to produce death by this treatment was from 1.0 to 4 mg. per gram of body weight for chicks and 0.5 to 1.5 mg. per gram of body weight for laying hens.

Studies of acute toxicity have been made by feeding pure gossypol obtained from the Southern Regional Laboratory, New Orleans, in capsule form to young chicks and laying hens. Intramuscular injections of pure gossypol, dissolved in Wesson Oil or suspened in Tween-80, were made into young chickens and into hens.

Pure gossypol, fed by capsule to 5 week-old chickens produced death within 103-118 hours in one instance when fed at 4.8 mg. per gram of body weight. In older birds, capsules containing from 4.0 to 6.0 mg. per gram of body weight also caused death within this period. Injections of pure gossypol into the breast muscle of chickens caused death within 103-118 hours when given 1.9 to 2.1 mg. per gram of body weight. In both the capsule feeding and injection studies, feed and water intake was reduced and a hemocentration resulted.

Day-old chicks were placed on a synthetic diet containing 0.4% free gossypol for a period of 18 days. The diet was then changed to a practical diet without gossypol. The liver and spleen of chicks sacrificed on the 19th day contained the ceroid pigment. Chicks sacrificed on the 5th and 15th days after gossypol was removed from the diet, showed a progressive decrease in the amount of pigment in the liver, and by the 24th day afterward, essentially no pigment was found.

Extensive necrosis was found in the pectoral muscle following injection of the Tween-80 gossypol suspension and the Wesson Oil solution of gossypol. After 48 hours, the muscle contained macrophages filled with yellowish-brown pigment. A minimal amount of necrosis was observed following Tween-80 or Wesson Oil only injections in control birds, but no pigment was found. Histochemical stains indicate that the ceroid pigment found after injection is the same as that resulting from the injection of gossypol. The pigment is phagocytized locally by macrophages, accumulates in the liver following injection and slowly disappears. The mechanism of removal is not known; although some of the pigment enters the biliary tract. It is suggested that lethal effects of gossypol may result from the action of the compound on vital tissues such as cardiac muscle and erythrocytes.

Preliminary biochemical studies have been made on the effects of gossypol on oxygen comsumption, using the Warburg apparatus. The in vitro addition of gossypol acetate solution to chick embryo homogenates resulted in a progressive decline in oxygen uptake with increasing dosages of the ester. In a second experiment, homogenates of the liver of hens fed capsules containing pure gossypol were used to determine the cytochrome oxidase activity. No difference was found in cytochrome oxidase activity between the capsule fed birds and controls with the concentrations used in this preliminary experiment.

These studies indicate that the toxicity of pure gossypol is less than that of pigment glands. Gossypol acetate inhibited the overall oxidative metabolism and cytochrome oxidase does not appear to be altered by the acetate form. A survey of the enzyme systems which may be involved in gossypol toxicity should be undertaken. In addition, the nature of other toxic substances in gossypol glands should be investigated and analyses of the ceroid pigment should be made. Thus, it is apparent that much additional research in this field is indicated.

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Discussion:

Q. What is the ceroid pigment.

A. It is a brownish-yellow pigment that is found in the livers and in the fatty tissue of birds dosed with gossypol.

Q. Did you make platelet counts before and during dosing with gossypol.

A. Yes. No differences were observed.

Q. What was the reason for the difference of food intake by birds given gossypol.

A. There is no explanation except that the birds just stopped eating and drinking.

Q. Was the effect the same on chronic and acute dosing with gossypol.

A. Birds given an acute dosage of gossypol, sometimes stopped eating for a few days, but thereafter, regained appetites. At a 4.4% acute dosage level of gossypol, the appetites of birds did not seem to be affected. In the case of chronic dosing with gossypol, the birds stopped eating and drinking.

Q. Was the possibility of gossypol changing in the rations considered.

A. Yes, we were aware of the "disappearance" of gossypol in stored rations. For that reason, we administered gossypol or the separated

cottonseed pigment glands to the birds by capsules and made certain the capsules got into the chick's stomach.

Q. Do you have any explanation for the protein effect on gossypol toxicity.

A. The gossypol toxicity is decreased by increased protein in the ration. We had a graduate student who was studying this phenomenon. He had some evidence that indicated gossypol was detoxified by combination with the protein. There are many angles to be considered in this protein-gossypol effect. Dr. Lyman has some data on ultracentrifuge studies that suggest the crosslinking of protein with gossypol. He will present this data later in the meeting.

Q. Were high protein levels studied.

A. Levels as high as 40% protein in the rations were used. The Experiment Station in Glendale, Arizona, in studying the toxicity of gossypol for chicks found isolated purified gossypol to be more toxic than a similar amount of gossypol given as pigment glands or a similar amount given as free gossypol in cottonseed meal. At levels of 0.016% and 0.020% purified gossypol in the chick ration, the chick growth was definitely depressed. The gossypol of pigment glands, at the same percentage level of gossypol, did not retard growth.

Q. What was the source of pigment glands used.

A. They were received from Dr. Lyman.

Q. Is it certain that gossypol was more detrimental than pigment glands.

A. Yes.

Q. Was the gossypol pure.

A. Yes. It was very pure.

PANEL ON COTTONSEED MEAL IN BROILER RATIONS

H. E. Robinson, Presiding

REPORT ON ANALYSIS OF DATA FROM COOPERATIVE BROILER EXPERIMENTS

V. L. Frampton
Southern Utilization Research and Development Division
New Orleans, Louisiana

A planning conference was held at the Southern Regional Research and Development Division three years ago to develop plans for additional collaborative research on the use of cottonseed meals in broiler rations. Many of those present participated in that planning conference and will remember that the view which prevailed then was that an experiment should be set up with the objective of demonstrating that (1) the nutritional quality of cottonseed meals could be defined in terms of the solubility of the meal protein in 0.02 Normal NaOH solution and in terms of the free gossypol content of the meals, (2) a mixture of a good cottonseed meal and soybean meal would provide a better protein supplement in broiler rations than either cottonseed meal or soybean meal alone, and (3) that prepress solvent meals were superior in broiler rations.

A partial report of the results of the collaborative experiment was presented at the conference held at the Southern Regional Research and Development Division in New Orleans two years ago. It was evident, from the results reported then, that the objectives of the collaborative experiment were not met in that it was demonstrated that good cottonseed meals are not necessarily defined in terms of protein solubility and free gossypol, that a mixture of a good cottonseed meal and soybean meal is not necessarily better as a protein supplement for broilers than either meal alone, and that the method of processing cottonseed does not determine whether the meal produced will be of good or poor nutritional quality.

It was noted at that time that the data from the collaborative experiment supported several important conclusions. Included among these was the conclusion that the simple correlation between growth and free gossypol, in the range of concentration occurring in the meals used, is small. The conclusion was also reached that the alignment of the rations in terms of their nutritive qualities was the same at all stations.

A more complete report is presented now of the results of that collaborative experiment. Although the completed computations in the statistical analyses are not available, a very good notion of the final conclusions which will be reached may be obtained from the analyses being reported.

Nine cottonseed meals were used in the collaborative study. These were blended into 27 rations. About 12,000 broilers were used in the experiment. Nine of the rations contained only cottonseed meal as the protein supplement, nine contained protein supplements in which 25% of the cottonseed meal was replaced by soybean meal, and nine of the rations contained protein supplements wherein 50% of the cottonseed meal was replaced by soybean meal. The replacements were on a nitrogen basis. One ration, with soybean meal as the protein supplement, served as the reference ration.

The rations were fed at several stations in three replicated experiments, to lots of 20 birds each, composed of ten pullets and ten cockerels. Records of individual gains and of feed consumption were kept.

Chemical analyses of the meals and rations were carried out at the Southern Utilization Research and Development Division. These analyses included the determinations of many meal and ration constituents, but only six of these factors seemed to be related in any important way to the growth response and to the feed efficiencies shown by the broilers on the rations. These chemical factors were total lysine, epilson amino lysine, total gossypol, energy, crude fiber, and arginine. Free gossypol, in the range of concentration fed in the rations, was not found important.

One of the cottonseed meals was processed in the presence of aniline. The rations prepared from this meal were omitted from the mathematical analyses because reliable determinations of lysine and of gossypol were not obtained. The ration containing soybean meal as the sole source of protein supplement (Ration CM50) was also omitted from the mathematical analyses because the data from the soybean meal-fed broilers would tend to force correlations and to obscure the effects of variables in cottonseed meal.

The data from only one station (Louisiana State University) are included in the calculations contained in this report. This station was selected because all of the replicated experiments were run simultaneously, and the variance between replications was no greater than the variance within replications. The average weights of the cockerels at eight weeks were used in the studies.

Simple correlations between growth and each of seven independent variables were calculated for the data from Station 3 (LSU). These calculations are recorded in Table 1.

Simple coefficient correlations calculated from the data at Stations 0, 3, 5, and 9 for ration series 1 are recorded in Table 2.

It must be pointed out that more than two factors are involved in these simple correlations, and therefore simple correlations of this

Table 1. Simple Coefficient Correlations Between Growth, Ration, and Meal Characteristics as Calculated for Data from Station 3 (LSU).

Constituent	Ration Series 1	2	3
Free gossypol	-.14	.00	-.03
Total gossypol	-.69	-.58	-.82
Total lysine	.87	.91	.06
Energy	-.39		-.78
Crude Fiber	.39	.54	.78
Arginine	.39	.87	.58
Epsilon amino lysine contributed by cottonseed meal	.81		
Total lysine contributed by cottonseed meal	.87		
Protein solubility in cottonseed meal	.60		

Table 2. Simple Correlations Between Growth and Chemical Factors for Ration Series 1 at Four Stations.

Chemical Factor	Station 0	3	5	9
Espilon amino lysine contributed by cottonseed meal	.87	.81	.78	.82
Lysine in ration	.67	.79	.80	.85
Total lysine contributed by cottonseed meal	.98	.87	.87	.89
Total gossypol	-.68	-.69	-.77	-.70
N solubility in cottonseed meals	.58	.60	.65	.74

type may be highly misleading and very frequently valid conclusions regarding the relative importance of any one factor may not be reached from consideration of simple correlation coefficients alone.

A regression study was carried out for the regression of growth of males at eight weeks on total lysine of the ration, total gossypol, crude fiber, energy, and arginine, and a regression equation for each of the ration series (e. g., Ration Series 1, 2, and 3) was determined. (Note: Ration Series 1 contained only cottonseed meal as the protein supplement; Ration Series 2 contained 75% cottonseed meal and 25% soybean meal, Ration Series 3 contained 50% cottonseed meal and 50% soybean meal.) The regression equations are:

for Ration Series 1, $G = -4290 + 6544\, l - 1963\, g + 321\, z - 618\, \Phi$

for Ration Series 2, $G = 553 - 33.4\, l + 1.91\, z + 224\, \Phi$;

and for Ration Series 3, $G = 100\, l - 800\, g + 9.2\, z + 480\, \Phi$

where G is the average weight of the cockerels at eight weeks, l is the total lysine in the ration, g is the total gossypol, z is the crude fiber, and Φ is the arginine.

There are not enough data available from consideration of Station 3 (LSU) alone to establish the degree of precision for the partial regression coefficients, but some idea of their relative importance may be obtained.

The relative contribution made by each constituent studied in the regression on the growth of the broilers in Ration Series 1 are recorded in Table 3, while those for Ration Series 2 are recorded in Table 4, and those for Ration Series 3 are recorded in Table 5.

Table 3. Relative Contributions of Chemical Factors to the Growth of Cockerels for Ration Series 1 at Station 3.

Meal	Total Lysine	Total Gossypol	Crude Fiber	Energy	Arginine
13	5026	-618	1352	0	-1124
16	5504	-779	1392	0	-1126
21	5503	-432	1348	0	-1279
36	5582	-744	1213	0	-1359
45	5750	-512	1458	0	-1299

Table 4. Relative Contribution of Chemical Factors to Growth of Cockerels for Ration Series 2 at Station 3 (LSU).

Meal	Total Lysine	Total Gossypol	Crude Fiber	Energy	Arginine
10	-28	±	12	0	408
13	-28	±	10	0	389
16	-30	±	10	0	408
21	-32	±	10	0	449
36	-30	±	9	0	430
45	-30	±	11	0	406

Table 5. Relative Contributions of Chemical Factors to Growth of Cockerels for Ration Series 3 at Station 3.

Meal No.	Total Lysine	Total Gossypol	Crude Fiber	Energy	Arginine
10	93	-125	381	0	826
13	95	-132	401	0	835
16	96	-163	406	0	820
21	95	- 91	411	0	845
36	96	-152	357	0	873
45	96	-105	417	0	878

It will be noted that in Ration Series 1, lysine is limiting and the growth is strongly dependent upon the lysine content of the rations. The total gossypol is of importance, while the energy of the rations is high enough so that variations in this factor were not reflected in the growth response. Crude fiber is highly beneficial to the broilers, and ariginine is in imbalance. Lysine supplementation in these rations should have a beneficial effect, while arginine supplementation should not.

In rations of Series 2 , where 25% of the cottonseed meal is replaced by soybean meal, lysine is no longer highly critical, and arginine is limiting. Crude fiber is still beneficial to the broilers. The effects of gossypol are obscured in these determinations.

In Ration Series 3, lysine is not critical, while arginine is limiting. Gossypol is important as a growth inhibitor, while crude fiber is still beneficial.

It is apparent from these calculations that the amino acid balance in broilers is important, and that it may be as important, or perhaps more important, than total gossypol in influencing the growth of broilers. It might be expected that lysine supplementation would be beneficial in the rations where the lysine level is in the range of 0.77-0.85%. Arginine appears to be in imbalance at this lysine concentration. Lysine is no longer critical in the range 0.85-0.90% while at this lysine concentration, arginine, in the concentrations appearing in the rations, is limiting.

Lysine in the concentration range .90 to 1.0 may be slightly in imbalance, while the arginine, at this lysine level, is even more critical.

These calculations are of importance chiefly in that they indicate the need for investigations to obtain data which will permit a more reliable definition of cottonseed meals that will be of optimum usefulness in broiler rations. Such studies should be designed to reveal the nutritional interdependence for broilers of all of the essential amino acids in cottonseed meals and to determine the processing procedures which will preserve the maximum usefulness of cottonseed meal for broiler rations.

Discussion:

Q. Are these data all on growth.

A. Yes.

Q. Are you discussing the effects of free gossypol or total gossypol.

A. Total gossypol. The coefficient of correlation for free gossypol is small.

Q. The statement was made that arginine may hurt the bird, on basis of statistical analysis of results. Can it be said that it does definitely.

A. All the variables that were present at time of this statistical analysis of the effect of arginine were not known.

Q. What are the rations.

A. Ration No. 3 had protein supplement composed of a 50:50 ratio of cottonseed meal to soybean meal; Ration No. 2 was a 75:25 ratio of cottonseed meal to soybean meal; Ration No. 1 contained cottonseed meal alone as the source of protein.

Q. Were studies conducted with standard protein of soybean in which no cottonseed meal was added to ration.

A. Yes, but the results of that study were not included in the statistical analysis.

Q. Does the statistical analysis include data from experiments in which the rations of cottonseed meal were supplemented with lysine.

A. No, enough data from those studies to make a statistical analysis were not obtained.

Q. Was the soybean meal the same in all rations.

A. Yes. A composite of five soybean meals was made and the composite was used as the standard protein source.

Q. In regard to the arginine imbalance of the other meals, would glutamic acid have overcome these differences by furnishing a transaminase system.

A. No answer can be given.

Q. Were the rations iso-caloric.

A. No.

Q. What was the range of crude fiber in the rations.

A. The lowest crude fiber content in the ration was 4.09%; the highest level was 6.30%.

Q. Was this the range in one series of tests.

A. This was the range of crude fiber in all rations tested.

Q. Does this mean there was a variation in the crude fiber content of each ration.

A. Yes.

Q. Was the coefficient of correlation for arginine negative with rations No. 1, 2, and 3.

A. It was negative with ration No. 1. It was positive in the case of rations No. 2 and 3.

Q. Would fiber have any effect when using small amounts of the 41% or 44% meal.

A. Fiber cannot have much effect until the 7% level is reached.

At Louisiana State University the effect of fiber in broiler rations was investigated. The effect of fiber was not noticed until the caloric intake of the bird changed. When ground rice hulls were used as a source of fiber, there was an indication of a fat-fiber reaction. The effect of this reaction on chick growth is one that should be investigated. It may be added that the fiber from hay is beneficial to chick growth.

Fiber was added to a fiberless cottonseed meal and a reduced digestibility was obtained in studies at Oklahoma State University.

If the rations are iso-caloric, there is no effect from fiber. At Louisiana State University the growth and digestibility for a series of iso-caloric rations were checked. Growth and digestibility were not affected even though fiber content was varied. But if the caloric content is changed, there will be a larger effect from fiber content. The fat-fiber interaction has been mentioned. With a 38% protein ration that contained 20% soybean meal and also cottonseed meal, fiber did not affect growth.

There is no basis for comparison of the two sets of data since one set pertains to data obtained from ruminant studies and the other set to data from chick studies. This shows that the results learned from studies with one type of animals cannot be used for prediction of the results that will be obtained with other types of animals.

PRESENT STATUS OF COTTONSEED MEAL IN BROILER RATIONS

Comments by:
H. L. Wilcke
Ralston-Purnia Company
St. Louis, Missouri

Cottonseed meal may be used in broiler rations during the first four or five weeks at levels up to a replacement of 50% of the protein from soybean oilmeal, provided that the gossypol level is within desirable limits. Data were presented showing no effect on

rate of growth on feed efficiency in broilers to five weeks of age when a cottonseed meal analyzing 42.2% protein, 4.2% fat, and 11.6% fiber was used to replace 20%, 33-1/3%, or 50% of the soybean protein in a practical broiler ration.

I would like to present some very recent data we have obtained. We fed the following rations to rats. The rations were iso-nitrogenous and iso-caloric.

Ration	Soybean Meal	20% C/S Meal 80% SB Meal	33-1/3% C/S Meal 66-2/3% SB Meal	50% C/S Meal 50% SB Meal
5 weeks weight	1.34	1.35	1.36	1.37
Feed efficiency	1.85	1.87	1.88	1.88
Fiber content	4.6	4.9	5.2	5.5

I think cottonseed meal of high protein content and low gossypol content may be employed in combination with soybean meal in broiler rations, provided economics of cottonseed meal permit this.

Comments by:
H. W. Bruins
The Quaker Oats Company
Chicago, Illinois

Research reported at these conferences two or three years ago pretty well define the specifications required for a cottonseed meal to be used in broiler feeds. However, in the past two years, the economics of the situation have not been favorable for the inclusion of cottonseed meal in broiler rations, except in California. To my knowledge, there is no use of cottonseed meal in commercial broiler rations east of the Rocky Mountains at the present time.

Since cottonseed meal has relatively more value in comparison with soybean meal for the ruminant than it does for the single stomach animal, it seems likely to us that requirements for cattle feeds are likely to bring the price up beyond what can be paid for cottonseed meal in poultry feeds. This will be particularly true as long as we persist in making cottonseed meals with high fiber and low protein content relative to soybean meal reducing the productive energy for poultry and swine.

My remarks will be very brief. Today very little cottonseed meal is being used in broiler rations except in California. When soybean meal is scarce in an area, cottonseed meal is then employed by feed manufacturers. The use of cottonseed meal is a matter of economics. If the price of cottonseed meal was lower and a high protein content meal available, then more meal would be used. I do not know how much cottonseed meal is being used in the southeast part of the United States. In areas where corn is available, mixtures of hominy and cottonseed meal are being used for ruminant feeding.

Comments by:
A. A. Heidebrecht
Western Cottonoil Company
Abilene, Texas

The limited use of cottonseed meal can largely be attributed to the following factors:

1. Fiber too high compared to soybean meal.
2. Protein too low compared to soybean meal.
3. Low lysine availability.
4. Variable quality of protein.
5. Gossypol toxicity.

In my opinion, cottonseed meal will never be used in large quantities in broiler feeds until the above factors have been solved or partially solved. Even though cottonseed meal might be offered containing 44% protein, there would still be considerable pressure against purchasing cottonseed meal because of the lysine and quality of protein problem.

Discussion:

Q. Dr. Wilcke, what was the protein content of the cottonseed meal used and what was the fat content of the rations.

A. The cottonseed meal contained 42% protein and 4.6% fiber. The rations contained 4.2% added fat.

Q. Dr. Heidebrecht, if the difference in price between 41% and 44% protein cottonseed meal was fifteen dollars, how much would be utilized.

A. Only a small amount. Not over 7.5% and more likely about 4.5%.

Committee on Planning
Cooperative Research on
Cottonseed Meal in Broiler Rations

Abstract of Remarks by:
J. R. Couch
Texas A & M College
College Station, Texas

Most of the work with cottonseed meal has been stated previously. There are many problems for future investigations. However, there is no need to engage in another broad collaborative feeding study with broilers as was conducted in the past years. Cottonseed meal can be added at the 15% and at the 20% level in broiler rations and good growth will be obtained. This has been established. The economics of cottonseed meal are preventing its increased usage in broiler rations.

Abstract of Remarks by:
H. W. Bruins
The Quaker Oats Company
Chicago, Illinois

I agree with Dr. Couch. More data is not needed about the possibility of adding cottonseed meal to broiler rations in order to maintain good growth. We do need some work on the effects of fiber and energy in the broiler ration. It is up to the cottonseed crushers to make efforts to produce a high protein content cottonseed meal and to make adjustments in technology so that the price of cottonseed meal will allow it to compete with other sources of protein in the market for broiler rations.

Abstract of Remarks by:
J. T. Raper
Dow Chemical Company
Lake Jackson, Texas

If sufficient data has been obtained from the past collaborative broiler feeding studies, the data could be placed in the IBM machines and a lot of useful information obtained. That data should include all facts about the meals and the rations fed.

Abstract of Remarks by:
A. B. Watts
Louisiana State University
Baton Rouge, Louisiana

More data are still needed. The present data cannot be extrapolated with confidence. There are many investigations to be made. As in ruminant nutrition where the digestibility of cellulose is so important, it is important in chick nutrition to know the amine acid availability pattern of a ration. With birds on a ration of 900 calories there is more retention of protein than with birds on a ration of 800 calories.

Nitrogen metabolism ought to be defined for the chick. Often times attempts have been made to extrapolate data from rat feeding studies to apply to chick feeding studies. What is in the rest of the ration that influences the assimilation and utilization by the chick of the protein from one ration more or less than that of another ration should be known. For the use of cottonseed meal in broiler rations basic information needed includes: (a) digestibility of the protein in ration; (b) availability to the chick of amino acids from that protein; (c) the pattern of amino acid distribution in a protein that is required by the chick to maintain good growth. Some basic research must be done to find facts that can be applied practically at a later date. Several years ago much work, basic fundamental research, was done on the protein and protein hydrolysates of corn.

Abstract of Remarks by:
V. L. Frampton
Southern Utilization Research and Development Division
New Orleans, Louisiana

This whole matter is a question of research and obtaining information. It should have nothing to do with the economics of cottonseed meal. From the previous collaborative broiler feeding study, one fact is offered to show that economics are not involved. The average weights of eight weeks' old cockerels fed cottonseed meal varied from 362 to 1106 grams. What caused this difference? The reasons for this great difference in weights should be determined.

Discussion:

Q. Dr. Watts, what type diets do you use.

A. We have used the practical broiler ration and also a semipurified diet. We should encourage people like Dr. Altschul to conduct basic research on the cottonseed protein molecule because of the importance of the arrangement of amino acids and their availability on chick nutrition.

Q. Dr. Frampton, I don't think the variation in weights of the chicks is due to the cottonseed meal factor alone. The environmental factor is an important one too. How many chickens does that data represent.

A. Sixty chickens.

Q. I think you would get the same variation in final weights if you used 1000 chickens. A lot depends on genetic and environmental factors.

A. These are average weights for two different cottonseed meals.

Q. Are the data for individual birds fed meals at one station or do they apply to the average weight gains of all birds fed meals at several stations. I believe one group here is talking about individual birds, and you are talking about the average weights.

A. The data are for average weights values. The average weights of birds on rations containing cottonseed meal CM-13 was 362 grams, while the average for birds on CM-45 was 1106 grams.

PANEL ON COTTONSEED MEAL IN RATIONS FOR LAYING HENS

H. L. Wilcke, Presiding

Collaborative Testing of AGU Method

V. L. Frampton
Southern Utilization Research and Development Division
New Orleans, Louisiana

The problems relating to the use of cottonseed meals in laying rations and the need for grading cottonseed meals for laying hens have grown from the fact that cottonseed meals have a tendency to promote discoloration in the yolks of eggs from hens receiving the meals.

One method, the AGU method, for testing the usefulness of cottonseed meals in laying rations was proposed by Dr. C. R. Grau. Interest in his method became general when economic conditions in California developed to make the use of cottonseed meals in laying rations profitable. Cottonseed meals have been used extensively in recent months for egg production in California. With the use of the method by the California State Quality Control Laboratory for grading cottonseed meals for laying rations in California, an independent collaborative evaluation of the method was deemed worthwhile.

The present report is based on the AGU results obtained from three of the seven collaborators involved in the testing. No data for color of egg yolks have been received.

Six cottonseed meals were used in the test. These were used at a level of 10% of rations made up as follows:

Cottonseed meal	10%
Soybean meal	15%
Ground yellow corn	15%
Ground milo	49.4%
Alfalfa meal	2.5%
Ground limestone	3.5%
Bone meal	3.5%
Manganized salt	0.5%
Source of riboflavin equivalent to 500 units per gram	0.3%
Source of Vitamin A, 2,250 units, Vitamin D, 300 units	0.3%

Cottonseed meal was replaced by soybean meal in the control ration.

These rations were fed at each station to a minimum of six laying hens that had not received any cottonseed product for a period of sixty days

prior to being placed on the experimental rations. Twelve eggs from each hen were collected starting with the tenth day after the hens were on the control ration. These served as the control; six were placed in cold storage for color evaluation after a period of six months, while six were used for AGU determinations.

The hens were then transferred to the cottonseed meal-containing ration; and twelve eggs were collected starting from the thirteenth day on the ration. Six eggs were placed in cold storage for color evaluation after the expiration of a six months' period, while six were used for immediate AGU determinations. The identity of each hen and egg was recorded.

The method used for determining the AGU values was that described by Dr. Grau (Gossypol-Cephalin Compound from Fresh Eggs of Hens Fed Cottonseed Meals. L. C. Woronick and C. R. Gray, Agri. & Food Chem., 3, 706 (1955)), with the exception that ten washings with acetone were used for removal of carotene and xanthophyll and other pigments from the yolk, rather than the five washings recommended in the paper cited. The use of the ten washings was recommended by Dr. Grau at a later date.

The cottonseed meals selected for the AGU Tests were shipped from Southern Utilization Research and Development Division to each station. These meals were selected to produce a wide range of egg yolk color, with the view of determining the correlation between AGU and intensity of egg yolk discoloration. One of the meals was an "egg-tested" meal from California, while one was a meal prepared at SURDD by extraction with acetone.

Chemical data for the meals are recorded in Table 1. It will be

TABLE 1

CHEMICAL DATA FOR COTTONSEED MEALS USED IN THE AGU TESTS

Meal	Type	Lysine %	Free gossypol %	Total gossypol %	Methionine %
100	Prepress	3.40	0.03	0.82	1.45
101	Screw-press	2.59	0.03	1.25	1.56
102	Screw-press		0.04	0.70	
103	Lab. prepn. acetone	4.35	0.08	0.27	1.65
104	Prepress	3.12	0.02	0.97	1.60
105	Prepress	3.07	0.02	0.66	1.50

lysine value for the meals (as determined using the 2,4-dinitrofluorobenzene method) ranged from 2.59 to 4.35. The lysine data may serve as an indication of the severity of the processing conditions used in the preparation of the meals. The free gossypol ranged from 0.02% to 0.08%; the total gossypol from 0.27% to 1.25%, while the methionine varied from 1.45% to 1.65%.

The data for average AGU values that have been reported up to this time from the three stations are recorded in Table 2.

TABLE 2

AVERAGE AGU VALUES FOR COTTONSEED MEALS REPORTED FROM THREE STATIONS

	Stations		
Meal	LSU	Anderson Clayton	Swift
100	.43	.31	.52
101	.61	.35	.52
102	.29	.20	.40
103	.50	.28	.52
104	.35	.20	.36
105	.30	.24	.29

An analysis of variance of the AGU data reported from one of the stations indicates that, although there is a marked variation in the AGU values calculated from the data obtained from individual eggs from a given hen, the differences between hens on a given meal are not significant. It also seems probable at this time that the AGU values calculated when all of the data are in will not permit a distinction between meals 100, 101, and 103 and between 102 and 105. It remains to be determined if there will be distinct differences in the egg yolk color after a six months' storage period.

Discussion:

Q. Was the actual ranking pretty much the same in different laboratories.

A. Yes.

Q. Did everyone take the readings at 445 mμ or at 440 mμ. One set of procedure directions called for making measurements at 445 mμ and another called for making it at 440 mμ.

A. Even if the readings were made by some workers at 445 mμ and by others at 440 mμ the difference in reading would be very slight.

Q. Can one get a brown colored egg yolk and have a negative AGU reading for the same egg. Eggs from hens fed meal no. 104 were dark colored even though the AGU reading on the egg yolk was almost zero.

A. It is possible to get a brown colored egg yolk and a very low AGU value for the same egg. This is due very likely to the oxidation products of gossypol in the rations. The AGU method does not measure the oxidation products of gossypol. It only measures gossypol-cephalin in the egg yolk.

Q. How much variation was there in the AGU values within one laboratory.

A. Dr. Grau will probably answer that question during his discussion of egg yolk discoloration.

Status of Egg Yolk Discoloration Research

Report by:
C. R. Grau
University of California
Davis, California

During the two years that have elapsed since the last conference on cottonseed meal, considerable progress has been made in applying some of the basic information that had been obtained previously. At least two cottonseed meal producers and two feed manufacturers have been testing and using meals by assaying them for contents of available gossypol. This assay procedure is based on feeding laying hens a diet containing 10% of the meal to be tested, and after eleven days, analyzing the egg yolks spectrophotometrically for gossypol-cephalin. By estimating the available gossypol, and expressing it as AGU (Available Gossypol Units), a standard has been established for "egg-tested" meal which contains less than 0.3 AGU. Such meals have been found to be safe for laying hens at levels up to 10% of the diet, when the criterion of safety was lack of objectionable darkening of yolks after three months storage at 35° F. When field trials on yolk discoloration were undertaken, it was considered important to have egg marketing experts as well as nutritionists observe the broken-out yolks, in order to establish realistic criteria for acceptability of yolk color. The egg-testing procedure does not detect gossypol oxidation products, some of which can cause yolk discoloration. These harmful oxidation products are generally not present in large amounts in meals, but they may be present at high levels in soapstocks,

especially those soapstocks that have been heated extensively with alkali to destroy gossypol.

The egg-testing procedure is laborious, and not well suited to simple quality control practice. Because no better alternative was available, however, one California oil mill investigated the method with laboratory and field trials, and began marketing for laying hens the portion of their production that met the standards for egg-tested meal. The economics of the California market situation have encouraged others to introduce testing procedures also.

In the application of new testing procedures, problems of variable results among laboratories, or within a single laboratory, are bound to develop. Some of the problems that have been encountered in estimating AGU have been solved, others remain, and no doubt still others will be encountered later.

Eventually the bioassay must be replaced by some simpler, chemical procedure. When meals very low in gossypol can be produced, either by using seed low in gossypol, or by chemical treatment of the meal, the hen bioassay will no longer be needed.

Results of Collaborative AGU Estimations: We have not yet completed all analyses of the six meals sent to various laboratories by SURDD, but our individual egg data available to date are given in Table 1. These results indicate the following ranking of meals as sources of gossypol, in decreasing order:

SURDD Meal Number	AGU (U. C. data)
101	0.42
103	0.32
100	0.26
104	0.26
102	0.18
105	0.17

The first two meals contain too much gossypol to allow them to qualify as "egg-tested." The last two could be fed safely even at 15% of the diet.

Other Informal Laboratory Exchanges: One lot of egg yolk was mixed at Fresno, and samples of it were distributed to seven laboratories throughout California. The results (Table 2) show some variation, but with one exception, fairly good agreement among laboratories. The results of several other exchanges of analysts, reagents, meals, diets, and eggs indicate that the major differences among laboratories can be attributed to hen differences. In a field trial, however, one laboratory found relatively little variation in AGU among five different strains kept under various conditions of management.

TABLE 1

Individual Egg Data for AGU for SURDD Meals

SURDD Meal No.	Hen No.	Available Gossypol Units (AGU) Laboratory A	A	B	Hen Average	Meal Average
100	305	0.20	0.23	0.26	0.23	
	327	35	25		30	
	487	14	26	30	23	.26
	489	25			25	
	393	25			25	
	368	34	30		32	
101	408	36			36	
	395	69	65	56	63	
	324	30		29	30	.42
	554	46			46	
	406	41			41	
	378	36			36	
102	555	13		25	19	
	492	18			18	
	344	04	14	09	09	.18
	486	19			19	
	377	21			21	
	350			19	19	
103	358	24	27		26	
	341	23	10	17	17	
	391	44			44	.32
	328	53	43		48	
	346	29			29	
	389	31			31	
104	355	33	37		35	
	379	28			28	
	301	29			29	.26
	496	24			24	
	375	17	15	16	16	
	376			21	21	
105	364	09	08		08	
	420	23			23	
	362	27	28		28	.17
	326	07	11	14	11	
	359	14			14	
	310	20			20	

TABLE 2

Collaboration Test of Composite Yolk Sample. Figures are Absorbance Differences (400-445 mμ).

Day of Analysis	7/22 Analyst		7/23 Analyst		
Laboratory	A	B	A	B	C
1	.036		.040		
2	.029	.018*	.030	.030	
3	.037	.038	.036	.027*	.036
4	.037	.037	.045	.045	
5	.052	.050	.049	.049	
6	.041			.045	
7	.038		.041		
Averages	.040		.040		

* Insufficient stirring; data discarded.

Studies with Glandless Cottonseed. Dr. Scott McMichael, who is working in developing strains of cottonseed low in gossypol at the USDA Crops Research Division, Shafter, California, furnished us with delinted seed for assay for available gossypol. We dehulled the seed, extracted it with hexane, and fed it to hens. The available gossypol in the samples varied from one strain to another, but two strains were almost devoid of gossypol (0.01 AGU and 0.06 AGU) compared with control seed treated in the same way (10.2 AGU).

Oxidation Products of Gossypol in Soapstocks. A spectrophotometric procedure for estimation of gossypol oxidation products has been developed, and compared with results of feeding various soapstock preparations to laying hens.

Report by:
B. W. Heywang
Southwest Poultry Experiment Station
Glendale, Arizona

Abstract: Mr. Heywang's remarks were brief. He indicated that when he fed egg tested meals to hens he got eggs that discolored on storage.

In his opinion any amount of discoloration is objectionable and that either an egg is good or it is not.

Report by:
Biagio Piccolo and V. L. Frampton
Southern Utilization Research and Development Division
New Orleans, Louisiana

Studies on the Relationship Between Egg Yolk Discoloration and Cottonseed Meal Constituents

Cooperative studies to determine the causes of egg yolk discoloration in eggs from hens fed cottonseed meals have been carried on in cooperation with Mr. Burt Heywang at the Southwest Poultry Experiment Station, Glendale, Arizona. The present report is concerned with the progress that has been made up to the present time. The investigations reported here were designed to determine the relative potency of gossypol, gossypol derivatives, and other contituents of cottonseed meals in inducing egg yolk discoloration when they are fed to laying hens.

The development of the brown and greenish-brown coloration in the yolks of stored eggs from hens that have received rations containing cottonseed meal is attributed in the literature to the presence of "free" gossypol in the meals. The possibility that the "free" gossypol is the most important factor in cottonseed meals might very well be challenged on the basis of published data and on the basis of observations recorded in this report. It is also pointed out that it has not been possible in our work to separate gossypol from darkly colored egg yolks.

It may be noted by the way of background, that the color bodies in the discolored egg yolk are intimately associated with the protein in the yolk, and that the chromogenic material which is responsible for the egg color is a pH indicator. The material is dark brownish-green in alkaline media and is either yellow color or is colorless in an acidic media.

It is this property of the chromogen of shifting color with a change in the pH that is one of the important factors in the development of the dark colorations in eggs during storage. The yolk of a freshly laid egg is acidic, whereas the whites, or the albumins, of freshly laid eggs are alkaline. During the period of storage there is a tendency for equilibrium to be established between the yolks and the whites. The quantity of alkaline materials in the whites overbalances the acidic materials in the yolk, so that the net consequence, after equilibrium has been established, is that the entire egg is alkaline. It is when the yolks have thus become alkaline that the dark coloration occurs.

It is manifestly necessary, in the establishment of relationships between constituents of cottonseed meal and intensity of coloration in the yolk, that a quantitative and objective measure of all factors, including color, be obtained. It is for this reason that attention has been given to the development of a measure of egg yolk color.

It has not been possible for us to isolate gossypol, nor any product that was recognizable as a gossypol derivative, from the yolks of darkly colored eggs. The chromogen is adsorbed sufficiently tightly by the protein that we have had no success in separating the two. That is, it has not been possible to get a colored extract from yolks that would be useful as a means of measuring the intensity of the coloration. It was also not possible to obtain optically clear solutions of the colored chromogen-protein complex for spectrophotometric determinations of the intensity of the coloration.

In view of our inability to obtain solutions from egg yolks that would be suitable for spectrophotometric determinations, color photography was used as a means of getting a measure of color intensity. Advantage was taken, in these determinations, of the indicator character of the chromogen in the egg yolk, and all measurements were carried out at standardized pH values. The yolks were each divided into two portions, one of which was adjusted to a pH value of 4.6 and the other to a pH value of 10.4. (The reason for selecting standard pH values is that there is a natural variation in pH from yolk to yolk.) Each portion was then photographed with standard colored film under carefully standardized conditions. The transparent positives from these photographs were placed in the spectrophotometer and the transmission spectra determined in the visible region of the spectra. The spectra for a control yolk are shown in Figure I, while those for a discolored yolk are shown in Figure II. The optical densities are plotted as the ordinate and the wavelength as the absissa. The difference in the areas under the two curves is taken as a measure of the extent of coloration.

Several assumptions are made in using this procedure as a measure of egg yolk color. It is assumed, for example, that at a given pH the intensity of the coloration is directly proportional to the concentration of the chromogen. It is also assumed that the optical density of the light transmitted through the positive transparency is directly proportional to the intensity of coloration in the egg yolk.

The feeling is that the colored film will produce the color of the yolk as it is seen by the human eye and that the intensity of the coloration in the film is related to the concentration of the chromosphere in the yolk. There is the recognition, of course, that the character of the light transmitted through the film cannot be used to identify constituents in the yolk, but it is expected that the intensity of the light, at the various wavelengths that are transmitted through the film, would be related to the psychological response

one obtains in looking at the eggs. It develops that there is a slight shift in the color value with control eggs, as may be seen in Figure I, but this is small in comparison with the shift in absorption with eggs that has been obtained from cottonseed meal-fed hens, as may be seen in Figure II.

Cottonseed meals used in the experiments are listed in Table 1. Recorded in the table also are the data for the "free" gossypol, total gossypol, chemically uncombined gossypol, lysine, and methionine. ("Free" gossypol is defined by the analytical procedure developed at the SURDD and includes the value for the chemically uncombined gossypol plus small quantities of "gossypol-like" pigments. The "total" gossypol is defined by the analytical procedure for "total" gossypol, as developed also at the SURDD. The chemically uncombined gossypol is the gossypol that can be recovered from the "free" gossypol extracts of cottonseed meal.)

These cottonseed meals made up 20% of the rations fed laying hens by Mr. Burt Heywang. A portion of the eggs produced on the cottonseed meal-containing rations was shipped to SURDD for examination using the photographic method outlined above, while a portion was retained at Glendale for observation there. The identity of the individual eggs and the hens producing them were maintained.

All of the datahave not been assembled. However, the average value of the observed color values, determined by the photographic method for the eggs produced with several meals are recorded in Table 1.

A coefficient of correlation of 0.77 was calculated in a comparison of the color data recorded in Table 1, and color values estimated by Mr. Heywang on visual examination of the eggs after three months of storage.

An interpretation of these color data, in terms of the chemical data found in Table 1, is not simple. Simple correlations, however misleading they may be, are recorded in Table 2. It is noted that the only two factors that seem to be of any importance at all are the total gossypol and epsilon amino lysine. "Free" gossypol and the chemically uncombined gossypol seem to be of little, if any, significance.

The relatively high negative correlation between egg yolk color and epsilon amino lysine is probably of significance, not that lysine is involved, but as an indication that the intensity of color in the egg yolk is related to the severity of treatment of the meal during processing. In other words, the indications are that an artifact produced in the meals is responsible for a substantial part of the coloration in the yolks. The more severe the treatment, as indicated by a low epsilon amino lysine, the more color in the yolks.

TABLE 1. Analyses of Meals Used in the Egg Yolk Discoloration Investigation

Meal No.	Meal treatment	% Free gossypol	% Total gossypol	% Chemically uncombined gossypol	% E-Amino lysine	% Methionine	Color
CM 52	Commercial	0.03	1.25	0.011	2.59	1.55	8.79
CM 53	Commercial	0.02	0.66	0.006	3.07	1.50	6.77
CM 54	Commercial	0.04	1.11	0.014	3.42	1.55	7.09
CM 55	Commercial	0.04	1.40	0.017	3.53	1.65	7.55
CM 56	Commercial	0.06	0.97	0.028	3.38	1.55	7.26
CM 57	Commercial	0.03	1.07	0.009	3.40	1.70	6.84
CM 58	Commercial	0.02	1.00	0.008	3.64	1.55	6.67
CM 59	Commercial	0.02	0.83	0.004	2.70	1.60	6.62
CM 60	Commercial	0.02	0.97	0.003	3.12	1.60	8.50
CM 61	Commercial	0.03	1.24	0.015	2.52	1.65	8.96
CM 62	Commercial	0.45	0.93	-	3.75	1.60	
CM 63	Commercial	0.04	0.82	0.017	3.70	1.50	7.84
CM 64	Commercial	0.02	0.75	0.008	3.60	1.70	5.68
CM 65	Commercial	0.04	0.77	0.010	3.33	1.70	
CM 66	Commercial	0.03	0.75	0.016	3.48	1.55	
CM 67	Commercial	0.03	0.82	0.010	3.40	1.45	
CM 68	Hexane Extr.	0.80	1.26	0.790	4.21	1.80	
CM 69	Acetone Extr.	0.08	0.27	-	4.32	1.65	
CM 70	Isoprop. Extr.	0.001	0.70	-	3.46	1.75	
CM 71	Glandless	0.00	0.0	0.0	4.53	1.65	
CM 72	Commercial	0.14	1.45	0.040	4.39	1.45	
CM 73	Commercial	0.03	0.99	0.008	3.37	1.60	
CM 74	Aniline Treat.	0.008	0.39	0.03?	-	1.60	
CM 75	Commercial	0.04	0.76	-	-	1.60	
	Control						3.56

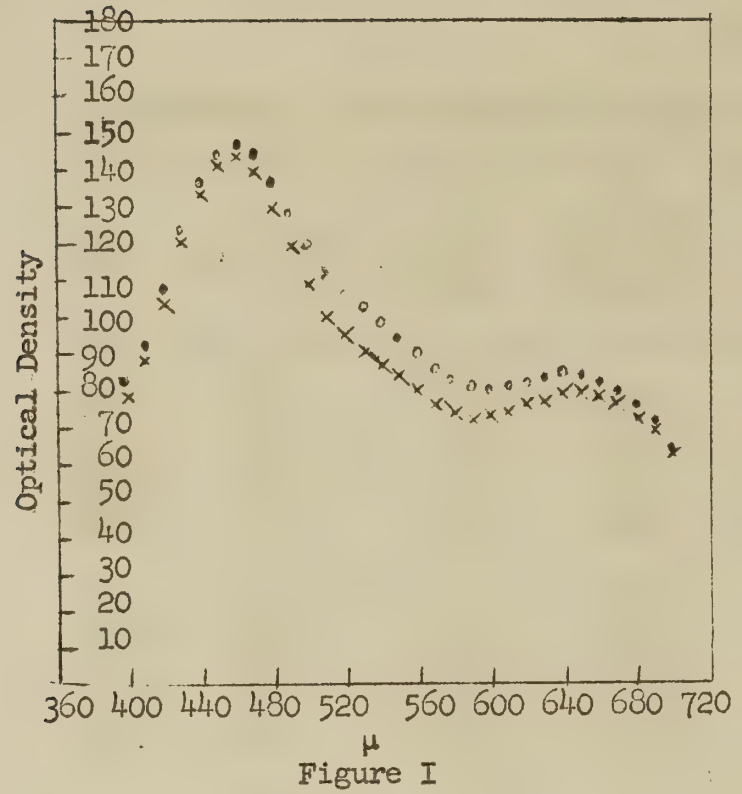


Figure I

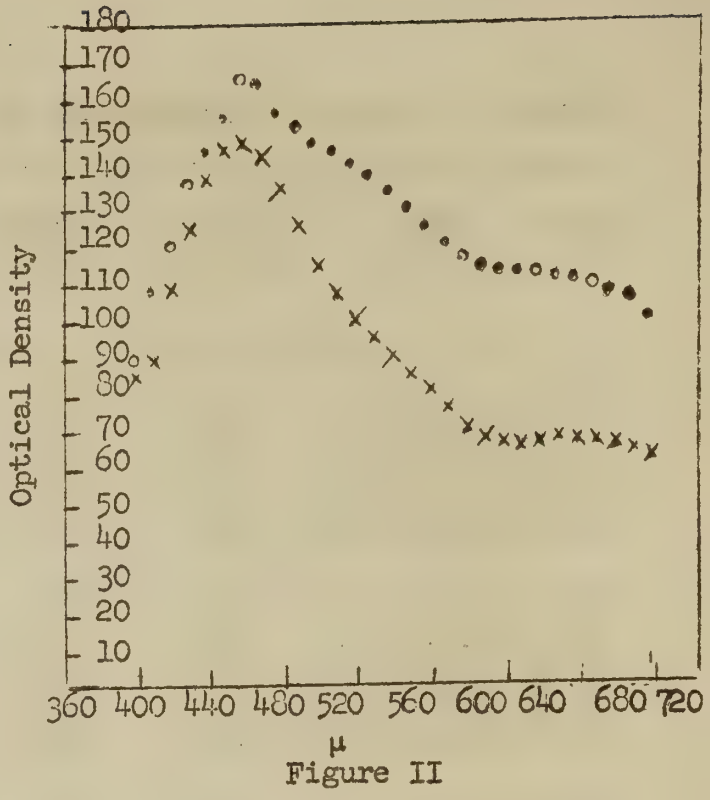


Figure II

Figure III

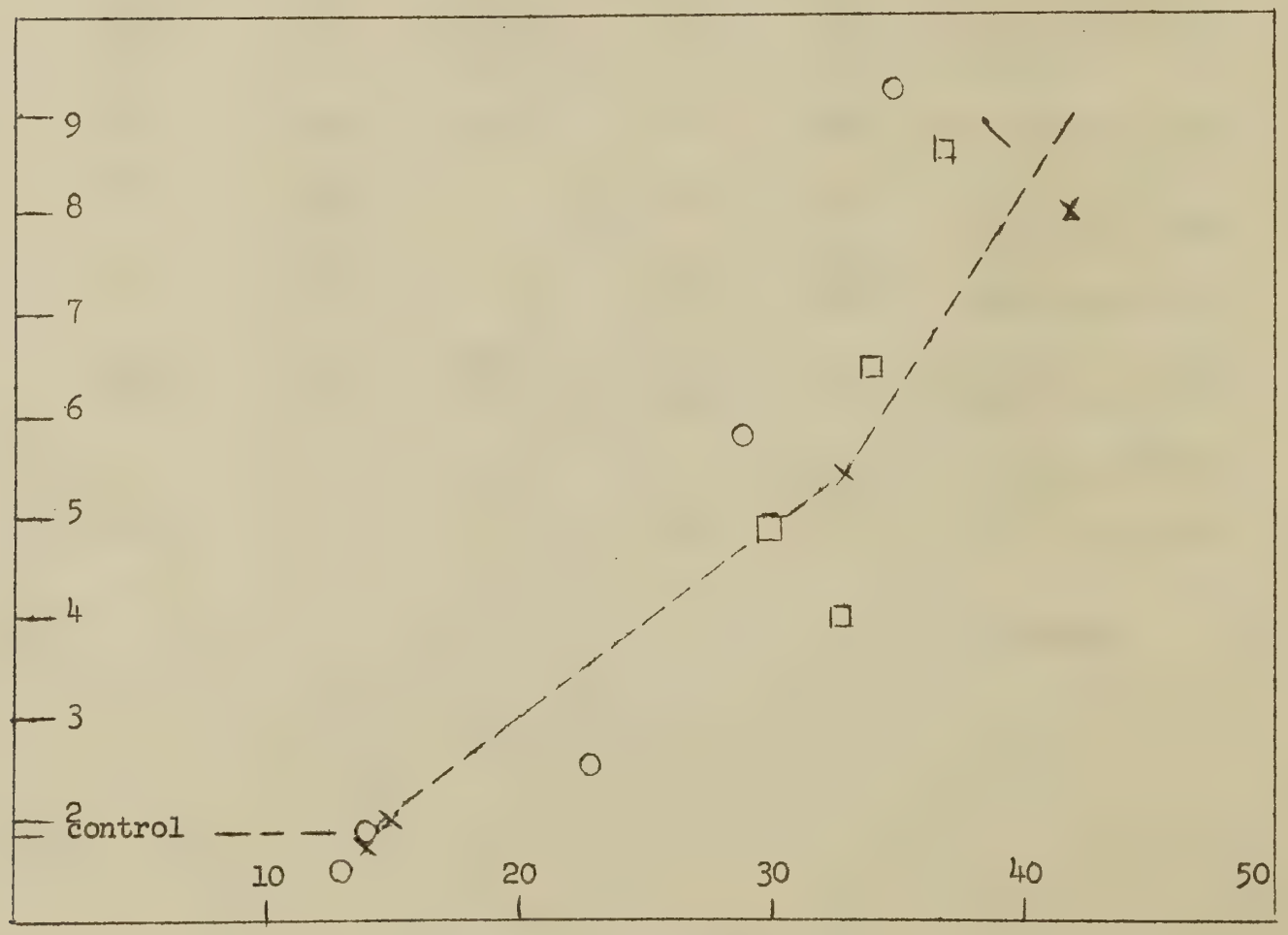


Table 2. Simple Correlations Between Egg Yolk Color and Meal Constituents

Free gossypol	0.19
Total gossypol	0.52
Chemically uncombined gossypol	0.01
Methionine	0.01
Lysine	-0.61

Two other observations that have been made in this investigation are of importance; the quantity of chromogen increases with storage time, and the quantity of chromogen in the yolk increases with time of feeding. Whereas no quantitative data on the effect of time of storage are reported at the present time, data recorded in Figure III indicate the effect of length of time of feeding cottonseed meal on the accumulation of the chromiferous materials in the hens. The effect noted in Figure III is general. It has been noted with every hen on the experiments where cottonseed meal was fed at the 20% level.

This effect of duration of feeding on cottonseed meal is of significance with reference to the grading of cottonseed meals for laying rations. It is not sufficient to examine eggs from hens during the first two weeks on the cottonseed meal diet - the observations on the eggs should be continued for a period exceeding 48 days before one can determine if the meals in question will induce discoloration in the yolks of eggs produced.

Discussion:

Q. With an AGU reading of 0.3, is the meal suitable for laying hen ration or is it thrown out.

A. The California ruling reads "not more than 0.3," so the meal is kept as a suitable feeding meal for laying hens.

Q. What was the variation for the AGU values of one meal.

A. The individual egg data for meal no. 101 ranged in values of: 0.36, 0.69, 0.30, 0.46, 0.41, and 0.36. Those are readings for one egg from each hen. With meal no. 105 the readings ranged as: 0.09, 0.23, 0.27, 0.28, 0.07, 0.11, 0.14, and 0.20. Reliable readings on yolk by the AGU method can be obtained. Some yolk were divided and seven laboratories assayed the yolk on two separate days. The laboratories duplicated each other in readings.

Q. How much yolk is used in making the test.

A. Ten milliliters. Samples from composites of yolks were sent to different laboratories.

Q. In determination of AGU are the differences due to chick factor greater than the differences due to manipulation of method by analyst.

A. The egg factor is important. With change in analysts no differences due to technician were observed.

Q. Have any studies been made on the effect of time between laying and feeding on egg colors.

A. No.

Q. Was any increase noticed in the AGU values of the eggs during feeding period with a particular meal.

A. No differences in AGU were observed.

Q. What number of hens are needed to test a meal.

A. A very poor meal or a very good meal will require only a few chickens. When the AGU value is on the borderline, that is, a value around 0.3, larger numbers of chickens are required for assay of meal.

Q. What is the variation among different strains of chicks.

A. Each hen is evaluated. Data for AGU's of control eggs for each hen are kept, and evaluated. A number is given to the readings for each hen and that number is then used in later determinations of AGU's.

Q. Would an egg of 0.3 AGU value that remained O. K. for three months, also remain O. K. for six months.

A. Yes, but eggs are not kept in storage for long periods in California, since ruling requires limitation on storage period. This is the risk in storing eggs from hens fed cottonseed meal. In California use is made of our cottonseed meal because soybean meal is so expensive there. There is a freight charge of $30 per ton for shipping soybean meal to the West Coast.

Q. If only two hens were used to test a meal, would the egg AGU values be averaged.

A. Each hen's eggs are evaluated on basis of egg AGU values before feeding the meal and after feeding it. In the case of very poor or very good meal the average value of each hen's eggs could be averaged to get final reading for AGU. At the time the AGU method was set up, no indication of the chick variation was known.

Miscellaneous Comments:

Some work on the egg yolk discoloration problem has been carried out at the Buckeye Laboratories. Good correlations between amount of gossypol in a meal and egg yolk discoloration were not obtained. The meal was then fractionated into three components. These components were: free gossypol; phospholipid derivative; and a hydrophilic fraction. The phospholipid substance was the one which gave the most pronounced egg yolk discoloration when added to the ration. The free gossypol, as measured by the chromatographic method of Schramm, was less pronounced in its ability to produce egg yolk discoloration. The third fraction, the hydrophilic fraction, produced relatively little discoloration. These three fractions should be equated in the matter of egg yolk discoloration. From them one can find out how much a meal will discolor eggs. If the hydrophilic fraction of the meal is high, then the amount of egg yolk discoloration produced by feeding it will be very low. It is felt that a study of meal fractionation along these lines may lead to many answers about this egg yolk discoloration problem.

Gossypol, that is, free gossypol, does not influence egg yolk discoloration. A hydroxyquinone may influence egg color.

The University of California tested several flavones. The ones found in the cotton plant include quercetin. These substances did not produce egg yolk discoloration when given to laying hens.

It is probably not the flavones but more likely the oxidation products of gossypol that cause egg yolk discoloration.

Q. In regard to the egg yolks that darkened on increase of pH, were the eggs more highly colored than control eggs before changing the pH.

A. Yes.

Q. When the rations were made with cottonseed meals, did the gossypol contents change during feeding time and while the rations were mixed.

A. The rations were fed as prepared. The reported gossypol values of the rations were the values for gossypol contents based on analysis of the meals at the beginning of the feeding study.

Q. What type variation was observed in eggs from hens fed cottonseed meal rations.

A. The colors of the egg yolks varied from dark yellow to light yellow to red to brown. The yolks also get larger the longer the hens have been on the cottonseed meal rations.

Commercial Use of Cottonseed Meals in Rations for Laying Hens

Abstract of Statement by:
A. H. Heidebrecht
Western Cottonoil Company
Abilene, Texas

For the present no cottonseed meal is being used in Texas for laying hens rations. This is due simply to the nature of the seed that is grown in West Texas. For example, the free gossypol content of meal from California ranges from 0.02 to 0.03 percent, while the free gossypol content of cottonseed meal from West Texas ranges from 0.03 to 0.10 percent. The Texas meal has an average free gossypol content of about 0.06 percent. The AGU values obtained with meals from West Texas are higher than those obtained with meals from California, even though the meals are prepared using the same processing conditions. The AGU value of Pecos cottonseed meal is 0.60; the AGU value of Lubbock cottonseed meal is 0.52. At this time the use of cottonseed meal cannot be recommended in rations for laying hens in Southwest Texas.

Abstract of Statement by:
B. F. Maxwell
Poultry Producers of Central California
Petaluma, California

The increase in cost of soybean meal and meat and bone meal in the spring of 1958 created an unprecedented interest in the use of cottonseed meal for laying hens. The egg merchandising firms find it cheaper to purchase 44% protein cottonseed meal than soybean meal for use in laying hens rations in California. The firms have set up egg testing units. AGU or other criterion are necessary if eggs of acceptable quality are to be produced. They have advocated the use of the egg test label or tag on the bags of cottonseed meal being produced in California. The bag of meal that carries an egg test tag is assumed not to exceed the 0.3 AGU value when the meal is fed as 10% of the laying hen ration. But of course, 5% level of a meal with AGU value of 0.6 would be the same using a 10% level of a meal with an AGU value of 0.3.

The test unit consists of 56 hens. A sample from one sack of cottonseed meal is fed to four hens. Ten sacks from each lot of meal are tested. This allows the firm to save money and does no harm to the eggs. When it is found that some eggs of a certain lot are more highly colored than others, the more highly colored eggs are collected and an effort is made to sell them without adding lightly colored eggs to the collection. For example, if a housewife buys a dozen of eggs that are of the same color even though the color may be darker than usual, she will not notice the egg discoloration as much as if she purchased a dozen eggs and found six of them to be of normal color while six were of much darker color.

Eggs from hens fed Ranchers' cottonseed meal have been stored for sixty and ninety days and discolorations have not been observed. Some have been stored as long as two years and showed no abnormal colors. Eggs obtained from commercial flocks on California cottonseed meal used according to AGU values have not shown any causes for complaint when placed in storage tests.

Statement by:
C. G. Cavanagh
Ranchers Cotton Oil Company
Fresno, California

Over 10,000 tons of Egg Tested Cottonseed Meal have been included in laying hen rations in California during 1958. This represents approximately 43,300,000 dozen eggs @ 65% lay. To date no objectionable discoloration of commercially stored eggs have been reported when cottonseed meal passing the Egg Tested requirements has been included up to 10% of the diet.

Protein solubility, free gossypol and AGU were determined on 45 commercially produced California cottonseed meals. Analyses showed there is a poor correlation between free gossypol and AGU. The so-called degossypolized meal is not necessarily safe for inclusion in laying rations.

A field test on three ranches using five strains of birds indicated that properly treated and processed cottonseed meal can be included in hen laying rations at least up to 10% of the diet. Storage tests for 3 and 6 months indicate a slight darkening of the yolk from feeding cottonseed meal. At 33° F. storage there were no problems with objectionably colored yolks after 3 and 6 months storage time. At 50° F. storage for 3 and 6 months, there were a few objectionably colored yolks.

Two commercial split flock feeding trials were run with essentially the same results as field trials on the three ranches.

Discussion:

Q. What causes mottling of eggs.

A. The mottling effect is due to storage of eggs at high temperature.

Q. Did the eggs have more discoloration the longer the hens were on the cottonseed meal rations.

A. No.

Committee on Planning
Cooperative Research on Use
of Cottonseed Meal in Rations
for Laying Hens

Instead of hearing statements from each of the Committee, most of the time was given to a general discussion among conference participants. Two problems associated with utilization of cottonseed meals in rations for laying hens were discussed. One problem is concerned with the egg yolk discoloration and the other with the pink albumins of eggs from hens fed cottonseed meals.

Discussion:

Q. Six meals were tested in the AGU study. How many were from California.

A. Only one meal was from California. It was meal No. 100.

Q. Was there any relation between AGU values and processing data.

A. Yes.

Q. Were all the eggs from hens fed meal No. 100 of an acceptable color.

A. When the meal No. 100 was fed at 20% level of the diet, some egg yolk discolorations were observed.

Q. Were the AGU values for eggs from hens fed meal No. 104 lower than the AGU values for eggs from hens fed meal No. 100.

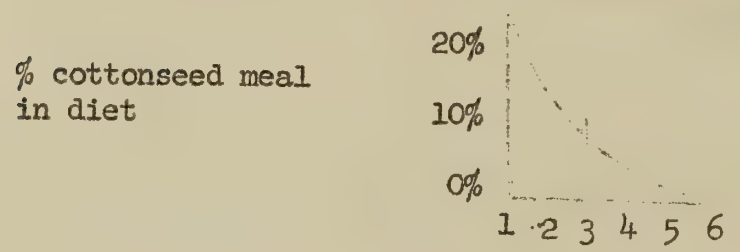
A. The AGU values of eggs from hens fed meal No. 104 were lower than those of eggs from hens fed meal No. 100.

Q. What processes were used for meal preparations.

A. Meals No. 100, 104, and 105 were prepressed, solvent-extracted meals. Meals No. 101 and 102 were screw-pressed meals. Meal No. 103 was prepared in the laboratory by extraction of cottonseed with acetone.

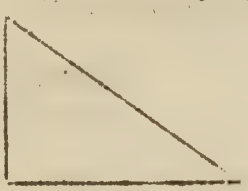
Q. Was any comparison made of meals at various diet levels.

A. Yes. One gets a series of curves such as this one:



Q. In determining AGU with meal at 2, 6, and 12% levels, do you get the same value.

A. Yes. It is essentially a straight line.



It seems there is an indication of the independence of cottonseed meal AGU value and percent cottonseed meal in the ration, with the amount of the factor responsible for AGU value in the egg. The AGU method of Grau must be understood in order to criticize or praise it. The method has nothing to do with the pink discoloration of egg whites. Some darker colored yellow eggs from hens fed cottonseed meals were obtained after eggs had been stored for six months, but the stored eggs did not contain red, green, or brown yolks.

The effect of carotenoids in the diet on egg color is significant. Care is taken of this by testing control eggs, i. e., collecting eggs from birds for a period before adding cottonseed meal to the ration.

If the AGU method does not measure all types of yolk discoloration produced by feeding cottonseed meal, then we must get methods that do.

Q. How can the photographic method be used by mills and feed manufacturers.

A. Charts will be made up. The charts will have color readings which the nutritionist or the miller can compare to egg colors.

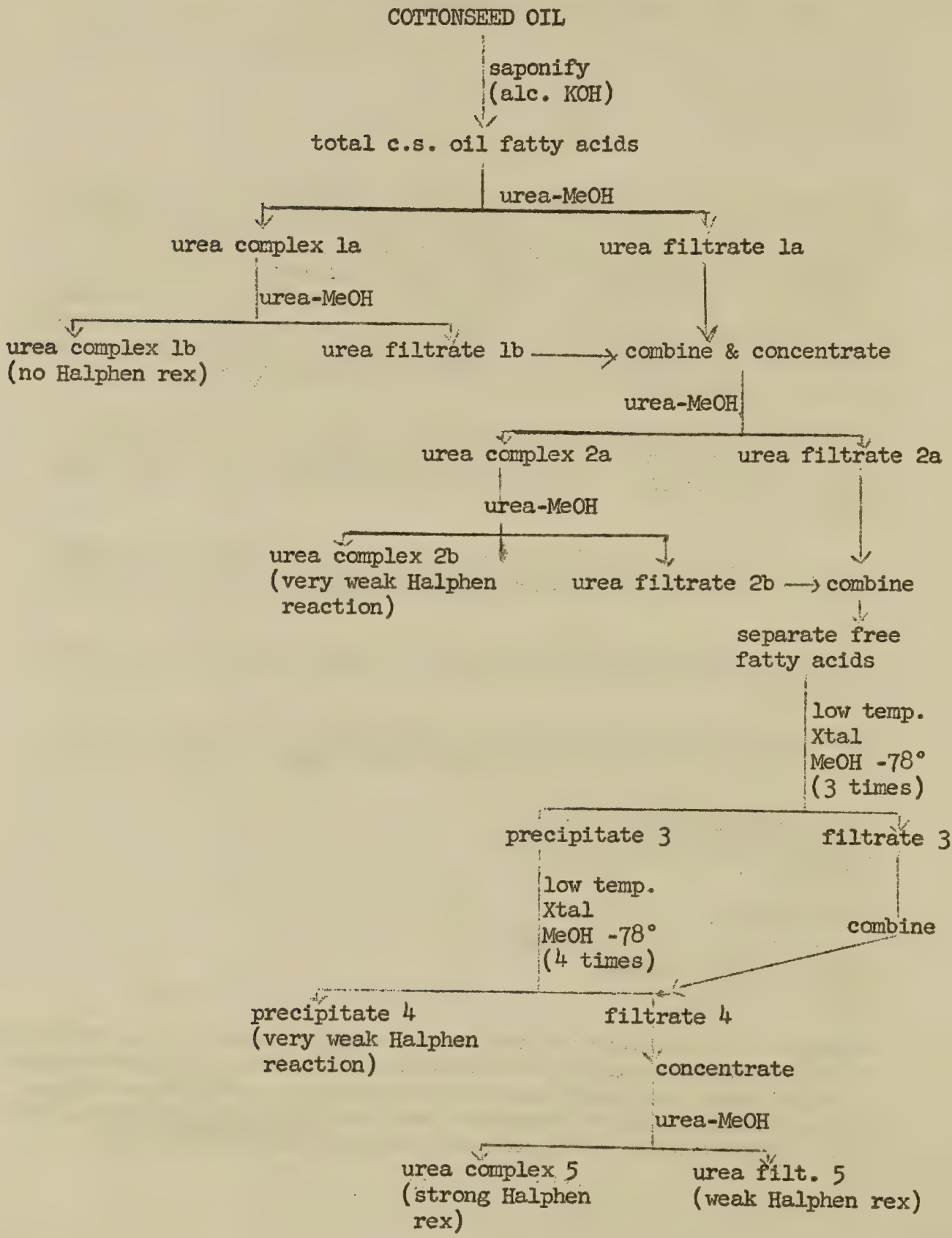
Pink Discoloration in Cold Storage Eggs Caused by Cottonseed Meal

Statement by:
James Masson and A. R. Kemmerer
University of Arizona
Tuscon, Arizona

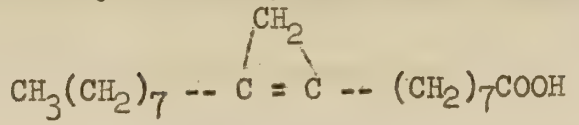
Abstract: From literature review and chemical tests it was speculated that the substance in cottonseed meal that caused pink discoloration in eggs might be a fatty acid which contained a cyclopropene ring. To test this hypothesis sterculic acid and Sterculia foetida oil were fed to laying hens. Both produced definite pink discoloration.

TABLE 1

Separation Procedure for Cottonseed Oil Fatty Acids



Sterculic acid is a cyclic fatty acid whose chemical formula is:



Fractionation of cottonseed oil indicates that a fatty acid of similar or the same structure is the factor responsible for the discoloration of of eggs caused by cottonseed meal.

Table 1 shows the procedure of fractionation of cottonseed oil.

Studies of Pink White Discoloration in Eggs

Statement by:
R. J. Evans
Michigan State University
East Lansing, Michigan

During the past two years we have studied the heat destruction of the Halphen acid in crude cottonseed oil and the isolation of the Halphen acid from crude cottonseed oil. Considerable concentration of this substance was achieved by crystallization of the mixed fatty acids from acetone in the cold. The Halphen acid was soluble at -60° C. but most of the other fatty acids were crystallized out at this temperature.

Crude cottonseed oil was heated at temperatures between 88° C. and 260° C. for times of a few minutes to 4 hours. Heating at 200° C. but not at 100° C. for 4 hours destroyed the pink white producing ability of the Halphen acid. The Halphen acid, as determined by the Halphen reaction, was destroyed by heating at 150° C. for 4 hours at 175° C. for 1 hour.

Yolk discoloration was variable and not entirely related to pink white discoloration. Further work should be done to study the interrelation of pink white and the different types of yolk discoloration.

Discussion:

Q. How much was the factor concentrated that gives positive Halphen Test.

A. About ten times original concentration in the oil.

Q. What was the content of sterculic acid to begin with.

A. It was less than one percent. Cottonseed meal containing less than 4% fat will give a positive Halphen Test. Degossypolized meal will give a positive test. Some work should be done on sterculic acid, to determine if it is the agent responsible for discoloration of egg whites, in cottonseed meals. A hydraulic cottonseed meal fed at levels of 10% and 15% of the rations, caused pink whites and green yolks of eggs.

PANEL ON USE OF COTTONSEED MEAL IN SWINE RATIONS

C. M. Lyman, Presiding

Status of Present Research:

Comments by:
I. P. Earle
Animal Husbandry Research Branch
USDA
Beltsville, Maryland

Abstract: A major objective in the studies carried out at Beltsville on the utilization of cottonseed meal in rations for growing swine has been to obtain some measure of the maximum amount of a meal of given characteristics which may be fed without injury to the weanling pig. Assuming that the toxic factor which limits the safe use of cottonseed meal in the pig diet is free gossypol, it would seem that toxic effects could be avoided by regulating the level of meal used according to its content of free gossypol, provided that the maximum amount of free gossypol which will be tolerated is known. However, it appears that the pig's tolerance for gossypol varies widely with other factors in the diet or with certain characteristics of the meal.

In studies of the gossypol tolerance of the growing pig, ten different meals have been tested. Most of these have been supplied by the SURDD and have been of known processing histories. All meals have been analyzed at the SURDD for total and free gossypol and for nitrogen solubility, and some of them for lysine as well. All meals have been assayed at Beltsville for protein quality by a rat repletion technic and many of them by a rat growth method also. The same general type of diet has been used in all tests, the principle variations introduced being in the level of protein, the content of free gossypol and, in the two most recent tests, in the use of supplements such as lysine to modify the quality of the protein. With one exception, all the meals studied have been low in free gossypol. Variations in the level of free gossypol have been obtained, in all except two tests, by the use of suitable small supplements of a special high gossypol meal containing by analysis either 0.6 or 0.8% free gossypol. In one test supplementary pure gossypol supplied by the SURDD was used and in another a blend of meals of different free gossypol contents was used.

Results of these tests have not indicated that either the general method of processing used in the production of the meals of the level of total gossypol in the diet was a factor in the toxicity of the diet. On the other hand, they have demonstrated that there is a definite relation between the level of free gossypol and the toxicity of a diet when other factors are kept constant, and between the amount of free gossypol which will be tolerated and both the level of protein in the diet and the quality or source of this protein. Levels

of gossypol which are well tolerated in 18 or 20% protein diets may be quite toxic when the protein is reduced to 13 or 14%. Meals which have a good biological value as assayed by a rat repletion method tend to be less toxic at a given gossypol level and a given protein level than meals of inferior biological value. This relation between rat repletion assay value of a meal and gossypol tolerance is, however, not always apparent, since in one test which utilized two meals of approximately the same medium assay value, one meal was toxic at a gossypol level as low as 0.009% while the other remained nontoxic at a level as high as 0.017%. This difference in the two meals is considered to be related to differences in the proteins which were not reflected in the response of the protein depleted rats.

Recently a test has been made of the effect on gossypol tolerance of lysine supplementation of the diet. Use has been made of a basal diet containing 13.5% protein and utilizing a prepress solvent-extracted meal which has been found highly toxic in a previous trial at a gossypol level of 0.011% in a 13% protein diet. A comparable level of gossypol was sought for this diet through the use of supplements of pure gossypol. This basal diet was supplemented for the different treatments with 0.1%, 0.2%, and 0.3%, respectively of l-lysine hydrochloride. Although the gossypol mixed in these diets was not recoverable as free gossypol, the basal diet without supplementary lysine was fatal to 2 out of 7 pigs. But there was no evidence of toxicity at any level of lysine supplementation although the daily intake of free gossypol per kilogram of body weight was slightly greater in these animals than in those without supplementary lysine.

This study of the effect on gossypol tolerance of amino acid supplementation of the diet is being continued.

Comments by:
A. J. Clawson, F. H. Smith, and E. R. Barrick
North Carolina Experiment Station
Raleigh, North Carolina

Cottonseed Meal Research with Swine

The effects of autoclaving cottonseed meal and lysine supplementation on gossypol toxicity were investigated in a trial starting with weanling pigs. Degossypolized cottonseed meal was used and gossypol from cottonseed meals was added to obtain gossypol levels of 0.02 and 0.03 percent in the diet. Three of 12 pigs receiving the 0.02 level of gossypol died and 10 of 12 receiving the 0.03 level. Autoclaving the meal markedly depressed rate of gain of pigs receiving 0.02% gossypol but did not influence death rate. Addition of 0.2% lysine to the diet containing autoclaved meal improved the performance to an extent that it was almost equal to the diet containing meal that was not autoclaved. Addition of lysine to a diet containing 0.03% of gossypol (meal not autoclaved) failed to improve performance or decrease mortality.

Experiments conducted to compare the toxicity of gossypol at levels of approximately 0.02% and 0.04% in diets containing protein from different sources have shown the following:

Addition of 0.02% free gossypol to corn-peanut meal or corn-cottonseed meal diets depressed performance but did not appreciably affect performance in corn-soybean meal, corn-fish meal or corn-meat meal diets.

Addition of 0.04% free gossypol depressed performance and resulted in high mortality in pigs receiving diets containing each of the different sources of supplemental protein.

Mortality rate was higher in trials conducted during winter months than in those conducted during summer months. This is believed to be associated with level of daily feed intake.

The method of Pons and Hoffpauir (Jo. Assoc. Off. Agr. Chemists , Nov. 1957, p. 1068) for determining gossypol in feed was investigated for determining the gossypol content of feces of pigs receiving 0.02 or 0.04% added gossypol. The method gave very promising results when the diets contained peanut meal, soybean meal or meat meal as a source of protein but the results were not consistent when degossypolized cottonseed meal was used as a source of protein.

Discussion:

Q. Dr. Barrick, was the 0.02% or 0.03% gossypol content the level of the diet or the meal.

A. It was diet level.

Q. Was gossypol added to degossypolized meal and autoclave mixture.

A. The meal was autoclaved and then gossypol was added to it.

Q. Is the free or total gossypol level being discussed.

A. The free gossypol level.

Q. What was the original gossypol content of the degossypolized meal.

A. The gossypol content was 0.024% to 0.025%.

Q. How many pigs did you test with each ration.

A. Three pigs on each trial. The following levels of gossypol were added to each type of rations: 0.000%, 0.016%, and 0.032% gossypol. The types of rations were: peanut meal ration, meat meal, fish meal, soybean oil meal, and cottonseed meal rations. With 0.02% gossypol in the soybean ration, there was observed better growth than with

ration of meal and bone that contained 0.02% gossypol. This indicates that one does not need the high-costing animal sources of lysine for swine growth.

Q. Were there any losses.

A. In the previous trial at 0.02% gossypol level there was one death. But in this trial there were no deaths at the 0.02% gossypol level. At the 0.04% gossypol level about one-third of the pigs died.

Q. For determining the total and free gossypol contents of the feces, what methods were used.

A. The recently published methods of Pons and Hoffpauir for determining free and total gossypol in mixed feeds were used. Amino-propanol was used as a complexing agent in these methods.

Q. What effect did autoclaving have on protein.

A. Autoclaving reduced nitrogen solubility of meal from 87% to 46%. It caused lowering of the lysine content from about 4% to 2.8%.

Q. Were the gossypol contents of the diets those obtained by analyses or by calculations.

A. By calculations.

Q. What is gossypol bound to in the feces.

A. According to testing methods, it was there as bound gossypol. The bound gossypol fraction has not been isolated to determine its components.

Comment: No analytical method is considered as the final method. When gossypol is added to feeds, the gossypol undergoes "disappearance." There is more to be learned about the relation of chlorophyll and other dietary components to gossypol in rations.

The meals rich in lysine showed no depreciation of swine growth. This should follow along with chick work. It appeared that lysine supplementation corrected to a surprising extent the growth depressant effects of gossypol, but it did not counteract gossypol toxicity. It is interesting to see how the growth rates stack up with the chemical characteristics of the dietary components.

Q. The data would indicate that swine are more sensitive to gossypol toxicity than chicks. The data from the chick studies show that free gossypol is not an important factor where growth is concerned.

Q. When did toxicity become apparent.

A. Tests were conducted for 82 days; most of pigs suffering toxic effects were dead within three weeks after beginning feeding trials.

Q. Dr. Earle, when you speak about adding gossypol to the swine rations, did you add gossypol or pigment glands.

A. Gossypol was added as a hexane-extracted cottonseed meal; the meal contains intact pigment glands.

Q. Dr. Couch, you added crystalline gossypol by capsule to the chick stomach. I do not think the gossypol was soluble and that is why you did not get any growth depression. In pigment glands the gossypol is water-dispersible and that could account for the differences in effect that you got with chicks.

A. The gossypol must have been adsorbed by the chick, even if it was crystalline, because it did cause death of chicks.

Q. Is it certain that the gossypol in the feces existed as free and bound.

A. The procedures of Pons and Hoffpauir were used. It was found that application of the methods to peanut meal or meat scraps without added gossypol gave a small test for gossypol. Of course, this value was deducted as a blank from the reading obtained after addition of gossypol to sample.

It is agreed that the methods for determination of gossypol in mixed feeds are not for determinations of gossypol in feces, but the methods can be applied to determination in feces with some small correction as you have mentioned. As we understand the experiment, you put free gossypol in the ration and recovered bound gossypol in the feces.

Q. Is it certain that the gossypol was not all free in the feces. Was the extraction prolonged to get out all free gossypol. Longer extraction may have gotten out more of the gossypol as free gossypol. The feces were dried before testing. That makes a difference. In the extraction of fresh feces, gossypol is free.

A. The method of Pons and Hoffpauir was applied. Extraction period for free gossypol was not prolonged. The feces were dried before determination of gossypol contents.

Comments: Perhaps this is tying in with the other work showing that when protein contains more lysine, there is more opportunity for the protein and gossypol to bind and help eliminate the toxicity of gossypol. Barrick showed that lysine did not entirely eliminate the

gossypol toxicity. But it is known that gossypol will combine with protein. Through studies such as Dr. Barrick's we get a better picture of what happens to gossypol in the animal body.

The question of free or bound gossypol in the feces will have to be subjected to tests with several methods. The interpretation of gossypol binding with the lysine of the protein and detoxification of gossypol by protein containing high lysine content is a good explanation, but the explanation is not so simple. The animal body is put under a nutritional strain when gossypol is part of the diet.

The amount of fiber in the diet will affect gossypol content of feces. For example, if fiber content is high, it will dilute feces and the gossypol content would be less than if the diet had been of lower fiber content.

The whole matter of detoxifying the meals by increasing protein quality of the diet is something more basic than just the combination of gossypol with lysine. We need to work on gossypol balance studies. There are two types of gossypol toxicity which have to be studied - chronic and acute toxicity. Mr. Heywang's data were on chronic toxicity studies, while the Texas data were on acute toxicities. Gossypol given by capsule to the chicks was adsorbed by the chicks. The gossypol had been adsorbed because it killed them and the ceroid pigment was deposited in livers and fatty tissues.

There must be emphasis made for more study of the metabolism of gossypol in the animal body.

The aldehyde next to the OH in pyridoxal, and similarity of that CHO next to OH in gossypol, makes one wonder whether gossypol acts as antagonist to protein combination with pyridoxal when both are at the same cellular level. This thought is one that should be explored to determine the action of gossypol in animal body.

While speculating about gossypol action, mention of the close relationship between gossypol and Vitamin K should be made. Then again one might think about Vitamin E and its action being repressed by gossypol.

Perhaps too much emphasis is being placed on gossypol. One can autoclave soybean meal alone and get reduction in chick growth.

The experiments on protein quality and toxicity studies with gossypol have shown that the toxicity of gossypol is a real and important factor in the cottonseed meal nutrition work.

When lysine was added back to the rations, there was some toxicity. Perhaps the gossypol-lysine complex is toxic.

Gossypol-lysine is inactive. But when it becomes biologically unstable, it may decolorize eggs. Perhaps this is the explanation.

Gossypol is a very strong acid. It dissolves in alkaline medium. It may combine with amino acids other than gossypol, and be solubilized in the animal body.

Schramm from Buckeye Corporation has developed a chromatographic method for determination of gossypol. He has isolated the crystalline gossypol from meals by this method. Even in the midst of a wide variety of gossypol-like pigments, this method is specific for gossypol and gossypol alone. It might be used for determining the gossypol content of feces.

Q. Dr. Earle, what gave the greatest toxicity: pure gossypol added to the ration or gossypol added in the form of cottonseed meal.

A. The gossypol of cottonseed meal was most toxic. This is in line with the findings of Dr. Couch that gossypol of pigment glands is more toxic than isolated gossypol. As mentioned earlier the meal used to add gossypol to the ration was a hexane-extracted meal that contained intact-pigment glands.

Q. Was l-lysine added.

A. l-lysine hydrochloride was added to the rations.

Comment: The picture of gossypol toxicity gets more complicated at each conference. The Georgia Experiment Station obtained evidence that points to other toxic materials in cottonseed pigment glands, i. e., other than gossypol. With swine the typical symptoms of gossypol poisoning such as edema, emanciation, and hemorrhagic gastro-interitis were observed. The thyroid and parathyroid appear to be involved in gossypol toxicity. Does anyone know what kills an animal poisoned by cottonseed meal.

Committee on Planning Cooperative Research on Use of Cottonseed Meal in Swine Rations

The members of the committee are of the opinion that more collaborative work on the utilization of cottonseed meals for swine rations is needed. The work should be similar to that being carried out to determine utilization of meal in rations for laying hens. The studies may center about these three approaches: (a) test 6 or 8 meals differing considerably in nitrogen solubility at two or more stations; (b) test the better of these meals at other stations and compare them to soybean meal for swine rations; (c) study the effect of lysine

supplementation of cottonseed meals. Dr. Lyman has been particularly interested in this phase of the work. The studies should include histopathological investigations of organs from swine fed cottonseed meals. Four groups indicated that their stations will collaborate in the swine tests.

Summary of Comments:

We cannot learn about the toxicology of gossypol and cottonseed meal poisoning from a feed and weight basis study alone.

It was suggested that bad results will be obtained because of the low lysine content of milo if milo and cottonseed meals are tested at a 16% protein level.

It was stated that no other study besides Dr. Barrick's is known that has been so complete and it was suggested the proposed lines of study should provide an excellent chance to establish the effects of gossypol with soybean meal rations for swine.

Two points were made in the discussion. One is that soybean meal produced better growth than fish meal in Barrick's study. The other is that Texas A & M shall be very happy to perform histopathological studies of tissues and organs from animals used in your studies.

It appears also that there is a serious need for study of the metabolism of isotopic-labeled gossypol.

The opinion was expressed that gossypol is causing all the trouble. The industry needs to get rid of it. Breeding gossypol-free cottonseed seems to be the answer to the problem. But if the efforts along those lines are not promising, the emphasis should be put on other lines of research such as the inactivation of gossypol by chemical means. It might prove worthwhile to look into the work that has been done with synthetic fiber systems. Try to tie up gossypol with a polyfunctional compound.

It was pointed out that there is considerable optimism about breeding of gland-free cottonseed.

The Subcommittee of the N.C.P.A. thinks the gland-free cottonseed will not be available in the immediate future, but feels that very early there will be made available to the industry a good planting seed of low free gossypol content.

Some workers are crossing Hopi seed, free of gossypol, with other seed to get a planting seed that still retains all the desirable characteristics for producing highest grade cotton. We cannot upset the genetic characteristics of cotton. For years we have been

working with cottonseed, and we have not known anything about the inherent factors of gossypol. Many workers thought there was some relationship in the seed between gossypol, oil, and protein content. Today for the first time we know this is not true. There is only a slight statistical correlation. Of course, it is necessary to find out whether gossypol is linked to other characteristics in the seed.

Since the seed is the byproduct of the cottonseed industry, it would be unwise to lower the quality of cotton to get gland-free seed.

If there is any secondary reduction in quality or yield of cotton, then the matter of gland-free seed is immediately out. We cannot consider any reduction in yield or quality of cotton. The cotton is the most important commodity.

Solving the gossypol problem of the seed would take care of the gossypol problem of cottonseed oil.

Three weeks ago there was a conference of plant breeders at this Southern Laboratory. In the past five years more advances have been made than those made during the past fifty years by the industry. Dr. Barker reported that gossypol is not linked with other characteristics (desirable characteristics) of the seed.

REPORTS OF COMMITTEES

Report of the Committee on Planning Cooperative Research for the Evaluation and Improvement of Cottonseed Meal in Broiler Nutrition

Considering previous research, the Committee again affirms the existence of sufficiently reliable information to guide processors in the manufacture of meals which can be effectively used in commercial broiler feeds. The Committee does recommend that cottonseed meal processors should further their application of this knowledge in plant operations. Likewise, because of the importance of energy in efficient broiler production, processors should be encouraged in the manufacture of the higher protein meals.

In analyzing the status of this phase of the research work on cottonseed meal quality, the Committee believes that at the present time greater progress can be achieved by stressing more basic fundamentals than by conducting collaborative practical feeding trials. It, therefore, recommends the following lines of basic research for the further evaluation and improvement of cottonseed meal in broiler nutrition:

1. Intensification of investigations of rapid assay techniques correlating cottonseed meal quality to chick growth tests.

2. Expansion of studies on the physiological effects of gossypol.

    a. Histopathology of chronic and acute toxicity of free, bound, and other forms of gossypol.

    b. Metabolic fate of gossypol and its derivatives as determined by:

        (1) Tissue evaluations.

        (2) Feeding and injection of isotopically labeled gossypol.

        (3) Other techniques which may prove feasible.

    c. Effects of other dietary components.

3. Studies on protein quality.

    a. Digestibility.

    b. Amino acid availability.

    c. Amino acid pattern.

d. Relationship of protein quality to gossypol content.

Harry J. Konen, Chairman
J. R. Couch
H. W. Bruins
J. T. Raper
H. E. Robinson
A. B. Watts

Report of the Committee on Planning Cooperative Research on Use of Cottonseed Meal in Rations for Laying Hens

I. Egg Yolk Discoloration

A. Status

The AGU (Available Gossypol Units) method of evaluating cottonseed meals for use in laying rations is under investigation to determine its general applicability. Evidence to date indicates that this method may be used satisfactorily in California with the type of cottonseed meals produced in that area and under marketing conditions where the egg storage period is less than three months. Further information is required before the AGU method can be recommended for meals produced in other areas or where different marketing conditions prevail. Cottonseed meal should not be used indiscriminately in laying hen rations even though the meal is "degossypolized."

B. Recommendations

1. The collaborative evaluation of the AGU method, including visual color evaluation, should be completed.

2. Information is required on what level of yolk color, particularly in stored eggs from hens fed cottonseed meal, is objectionable to the consumer.

3. Research is needed to determine if the degree of discoloration in yolks changes when a hen is on a discoloring cottonseed meal ration for a prolonged period.

4. Research is needed to determine the length of time necessary for a hen to consume a discoloring cottonseed meal ration before yolk color develops.

5. The components in cottonseed meal, as well as the factors in the egg responsible for egg yolk discoloration, should be identified; research on discoloring gossypol derivatives to be reported at the Gossypol Chemistry Conference in March should be extended to develop a chemical method for evaluating cottonseed meals for laying hens.

II. Pink Egg White Discoloration

A. Status

Pink white discoloration may be a problem when eggs from hens fed rations containing certain cottonseed meals are stored. Hexane extracted cottonseed meals containing less than 1% of fat will not cause pink egg white discoloration. (There was not unanimity in the committee on this point.)

B. Recommendations

1. Research is required to identify and to ascertain the physiological effect of the cyclic fatty acid(s) in cottonseed oil.

2. Methods of determining the cyclic fatty acid(s) in cottonseed oil and meal are needed.

3. Research is necessary to determine the level of the cyclic fatty acid(s) in cottonseed oil and meal necessary to produce discoloration.

4. Methods of eliminating the cyclopropene structure from cottonseed meals and oils should be investigated.

P. D. Aines, Chairman
C. R. Grau
B. W. Heywang
H. C. Wilcke
R. J. Evans
A. R. Kemmerer
V. L. Frampton

Report of the Committee on
Planning Cooperative Research on the
Use of Cottonseed Meal in Swine Rations

It is recommended that a collaborative swine feeding test be conducted with cottonseed meal with the following objectives:

A. To correlate performance of growing pigs with chemical characteristics of the meals, such as nitrogen solubility, total gossypol, free gossypol, amino acid composition (Moore and Stein method), tryptophane, methionine, lysine epsilon amino groups, and crude fiber.

B. To determine the effect of lysine supplementation with meals of different protein quality.

C. To compare cottonseed meal of high protein quality with soybean meal and mixture of cottonseed meal and soybean meal, 50-50, and 25% CSM-75% SBM, on protein replacement basis.

Procedures:

Eight different cottonseed meals with varying chemical characteristics will be fed in comparative feeding trials at two locations with corn as the grain and preferably a third location with milo replacing corn lb. per lb.

One of these meals of high protein quality will be compared with soybean meal and mixtures of CSM and SOM (50-50) and 25% CSM with 75% SOM on protein replacement basis.

A meal with high protein quality and one with low protein quality will be tested with lysine supplementation at two locations.

Dr. Barrick will confer with the collaborators and establish the composition of the rations to be used.

The meals to be used in these tests are to contain not more than 0.05% free gossypol.

The corn--CSM rations will contain 14% protein reduced to 12% protein when each lot reaches an average weight of 125 lbs.

Protein content of the different meals will be equalized by the adding of hulls.

Dr. Frampton will have charge of the collection and distribution of the meals and the coordination of the results.

Other Suggested Research

1. A study of the metabolism of gossypol in pigs using radioactive gossypol.

2. A study of the applicability of chemical determinations to balance studies on gossypol in pigs.

3. A study of the effect of gossypol in cottonseed meal on reproduction and lactation in swine.

4. Determination of availability of amino acids in cottonseed meal to swine.

A. A. Heidebrecht, Chairman
E. R. Barrick
E. A. Denton
K. T. Holley
F. H. Smith
C. M. Lyman
I. P. Earle

Discussion:

Q. Why use eight meals. Wouldn't it be better to forget meals of intermediate values and get more analytical data on fewer meals. With hog studies, it is not always possible to test sample in triplicate at one station. Wouldn't it be better to have complete study, i. e., as complete as possible, on fewer meals.

A. The Committee decided the least number of meals to be tested would be from six to eight. So it selected eight meals. Two groups will test four meals each. The better of the meals will then be tested at two other stations and be compared with soybean meal on a 50%, 25%, and 75% replacement basis.

Q. How many pigs will be tested with each meal sample.

A. Six pigs per sample will be used. Dr. Barrick is in charge of making the decisions on this collaborative work. His decisions will be final.

It was proposed by Leonard Smith of the National Cotton Council of America that the Conference recommend increased work on the chemistry of gossypol as such research is very important when the results of the Conference are brought to the attention of the Cotton and Cottonseed Research and Marketing Advisory Committee. This Advisory Committee has always recognized the importance of research on the chemistry of gossypol and its derivatives. Such work has been a part of the research program ever since these conferences were initiated. The following recommendation was proposed:

General Recommendation by Conference for Research

It is resolved that there is an urgent and immediate need for increased research on the chemistry of gossypol and gossypol derivatives, for increased research on chemical means for inactivation of gossypol and gossypol derivatives, and increased research on development of methods for the specific determination of gossypol and gossypol derivatives in cottonseed, cottonseed products, animal rations, and in animal organs and waste products.

The reports and recommendations of the Committees and the General Recommendation by the Conference for Research were approved.

Appreciation for attendance and participation in the Conference was expressed by T. H. Hopper and Garlon A. Harper for the Southern Regional Research Laboratory and the National Cottonseed Products Association, respectively.

Research Planning Conference on Processing as Related to Nutritive Quality of Cottonseed Meals

Sponsored by
National Cottonseed Products Association
and
Southern Utilization Research and Development Division

January 19-20, 1959
at
Southern Utilization Research and Development Division
New Orleans, Louisiana

January 19, 1959 -- Monday Morning

Conference Room
9:00 a.m. — T. H. Hopper, SU, Chairman

Opening Remarks ---------- G. E. Goheen, SU; Garlon A. Harper, NCPA; A. M. Altschul, SU

9:20 a.m. Purpose of Conference ---------- T. H. Hopper

9:30 a.m. Response of Ruminants to Cottonseed Meals of Known Quality as per Chick Test ---------- A. D. Tillman

9:45 a.m. Chemistry of Gossypol ---------- D. A. Shirley

Histopathological Effect of Gossypol ---------- J. R. Couch

10:00 a.m. (Coffee Break)

Panel on Cottonseed Meal in Broiler Rations ---- H. E. Robinson, Presiding

10:15 a.m. Report on Analysis of Data from Cooperative Broiler Experiments ---------- V. L. Frampton

10:30 a.m. Present Status of Cottonseed Meal in Broiler Production ---------- H. L. Wilcke; H. W. Bruins; A. A. Heidebrecht

10:50 a.m. Committee on Planning Cooperative Research on Cottonseed Meal in Broiler Rations ---------- H. J. Konen, Chairman

J. R. Couch, H. W. Bruins, J. T. Raper,
N. R. Lockmiller, H. E. Robinson, A. B. Watts

12:00 Noon Lunch

January 19, 1959 -- Monday Afternoon

Panel on Cottonseed Meal in Rations for Laying Hens ---------- H. L. Wilcke Presiding

1:00 p.m. Collaborative Testing of AGU Method ---------- V. L. Frampton

1:30 p.m. Status of Egg Yolk Discoloration Research ---------- C. R. Grau, B. W. Heywang, V. L. Frampton

2:00 p.m. Commercial Use of Cottonseed Meals in Rations for Laying Hens ---------- A. A. Heidebrecht, B. F. Maxwell, G. C. Cavanagh

2:30 p.m. Committee on Planning Cooperative Research on Use of Cottonseed Meal in Rations for Laying Hens -- P. D. Aines Presiding

C. R. Grau, B. W. Heywang, H. L. Wilcke,
R. J. Evans, A. R. Kemmerer, V. L. Frampton

January 20, 1959 -- Tuesday Morning

Panel on Use of Cottonseed Meal in Swine Rations --- C. M. Lyman Presiding

9:00 a.m. Status of Present Research ---------- C. M. Lyman, E. R. Barrick, E. A. Denton

9:30 a.m. Committee on Planning Cooperative Research on Use of Cottonseed Meal in Swine Rations ------ A. A. Heidebrecht Presiding

E. R. Barrick, E. A. Denton, K. T. Holley
F. H. Smith, C. M. Lyman, I. P. Earle

10:30 a.m. (Coffee Break)

10:45 a.m. Separate Meetings of the Three Committees for Preparation of Reports and Recommendations (Those attending the Conference who are not assigned to a Committee may sit in with one of their choice.)

12:00 Noon Lunch

January 20, 1959 -- Tuesday Afternoon

1:00 p.m. Reports of Committees on Plans and Recommendations for Cooperative Research ---------- T. H. Hopper Presiding

(a) Committee on Cottonseed Meals in Broiler Rations ---------- H. J. Konen

(b) Committee on Cottonseed Meals in Laying Hen Rations ---------- P. D. Aines

(c) Committee on Cottonseed Meals in Swine Rations ---------- A. A. Heidebrecht

Discussion and Action on Reports

Adjournment

APPENDIX

Attendance List

P. D. Aines..... Buckeye Cellulose Corporation, Ivorydale, Ohio
E. R. Barrick..... North Carolina State College, Raleigh, North Carolina
H. W. Bruins..... The Quaker Oats Company, Merchandise Mart Plaza, Chicago 54, Illinois
G. C. Cavanagh..... Ranchers Cotton Oil Company, Fresno, California
J. R. Couch..... Texas A & M College, College Station, Texas
I. P. Earle..... Animal Husbandry Research Branch, USDA, Beltsville, Maryland
E. A. Denton..... Animal Husbandry Research Branch, USDA, Beltsville, Maryland
R. J. Evans..... Agricultural Experiment Station, Michigan State University, East Lansing, Michigan
C. R. Grau..... Department of Poultry Husbandry, University of California, Davis, California
Garlon A. Harper..... National Cottonseed Products Association, 618 Wilson Building, Dallas, Texas
A. A. Heidebrecht..... Western Cottonoil Company, Abilene, Texas
B. W. Heywang..... Southwest Poultry Experiment Station, ARS, Glendale, Arizona
James Hickey..... Forest City Cotton Oil Mill, Forest City, Arkansas
K. T. Holley..... Georgia Agricultural Experiment Station, Experiment, Georgia
A. R. Kemmerer..... Agricultural Experiment Station, University of Arizona, Tucson, Arizona
Harry J. Konen..... 2472 Bolsover Street, Houston 5, Texas
C. M. Lyman..... Agricultural Experiment Station, Texas A & M College, College Station, Texas
B. F. Maxwell..... Poultry Producers of Central California, P. O. Box 298, Petaluma, California
R. A. Phelps..... National Cottonseed Products Association, 618 Wilson Building, Dallas, Texas
J. T. Raper..... Dow Chemical Company, Lake Jackson, Texas
H. E. Robinson..... Swift and Company, Union Stock Yards, Chicago 9, Illinois
D. A. Shirley..... University of Tennessee, Knoxville, Tennessee
F. H. Smith..... North Carolina State College, Raleigh, North Carolina
Leonard Smith..... National Cotton Council of America, Washington 6, D. C.
Staff Members..... Southern Regional Research Laboratory, New Orleans, Louisiana

Leah C. Berardi
F. G. Dollear
V. L. Frampton
G. E. Goheen
C. L. Hoffpauir
T. H. Hopper
Nestor B. Knoepfler
W. H. Martinez
R. M. Persell
Biagio Piccolo
W. A. Pons, Jr.

A. D. Tillman..... Oklahoma State University, Stillwater, Oklahoma
A. B. Watts..... Agricultural Experiment Station, Louisiana State University, Baton Rouge, Louisiana
H. L. Wilcke..... Ralston-Purina Company, Checkerboard Square, St. Louis, Missouri